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Identification and Epidemiology

RESEARCH

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ACADEMIC DISSERTATION

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To my family

Abstract

Leila M Sihvonen. Clinical Isolates of *Yersinia enterocolitica* in Finland - Identification and Epidemiology. National Institute for Health and Welfare (THL). Research 117. 145 pages. Helsinki, Finland 2014.
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Yersinia enterocolitica causes gastroenteritis in humans. Yersiniosis is usually acquired by consumption of contaminated foods. It is the third most common zoonotic bacterial enteropathogen in Europe and in Finland after *Campylobacter* and *Salmonella*. Yersiniosis is usually mild and self-limiting, however severe complications can appear, especially in certain high risk groups. Further, *Y. enterocolitica* infection can result in sequelae, such as reactive arthritis, which may become chronic.

Y. enterocolitica is a heterogeneous bacterial species, which can be invasive (pathogenic biotypes), or possibly entirely harmless as exemplified by many biotype (BT) 1A strains. *Yersinia* bacteria are able to grow at 4° C, *i.e.* refrigerator temperatures, which makes them a possible cause of food-borne disease. Identification in the laboratory, however, is challenging because *Y. enterocolitica* can be easily confused with related species – so called *Y. enterocolitica* –like species or environmental *Yersiniae* - which are considered mainly harmless to humans. Moreover, since *Y. enterocolitica* grows slightly slower than competing bacteria, it can remain undiscovered in test samples. Across Europe, differences in laboratory and reporting practices result in *Y. enterocolitica* infection being possibly underdiagnosed in some countries.

The purpose of this study was to investigate the diversity of clinical *Y. enterocolitica* strain subtypes, their sensitivity to antimicrobials and to find out the most suitable methods for isolating *Yersinia* from clinical stool samples, as well as to improve epidemiological typing methods. In addition, the clinical picture of the disease, the incidence of sequelae, as well as the source of the infection was investigated. Material for the work was collected in 2006 from 10 Finnish clinical microbiology laboratories with background information of the strains and methods used. Furthermore, a case-control study was conducted among the patients.

The invasive *Y. enterocolitica* strains possessing virulence plasmid pYV (pYV+) could be separated from non-virulent strains by phenotypic methods. Cold enrichment increased the yield of pYV+ strains from the clinical stool samples. A Multilocus Variable Tandem Repeat Analysis (MLVA) method was found to be a powerful epidemiological tool with higher discriminatory power and better

repeatability when compared with PFGE. Separation of *Y. enterocolitica* BT 1A strains from *Yersinia* enterocolitica –like species was difficult with phenotypic methods. Subtyping by the Multilocus Sequence Typing (MLST) method showed that the BT 1A strains were actually divided into two completely separate genetic groups. Moreover, the study found a virulence associated *ail* gene in two *Y. enterocolitica* BT 1A strains.

The symptoms of patients with *Y. enterocolitica* BT 1A differed from those of patient with strains of pYV+ BTs 3-4/O:3 and 2/O:9. A risk factor for a *Y. enterocolitica* infection with pathogenic BTs was consumption of raw or undercooked pork. Of these patients with pathogenic BT, 23% had travelled abroad before falling ill with gastroenteritis. Moreover, 19% of pYV+ *Y. enterocolitica* strains had developed resistance to four or more tested antimicrobials. The resistance to antimicrobials correlated strongly with travelling abroad. Children were over-represented in pathogenic BTs infections.

In conclusion, the use of cold-enrichment, Cefsulodin Irgasan Novobiocin (CIN) agar, and pYV plasmid detection was proved to be successful in isolating and identifying pathogenic *Y. enterocolitica* strains from human faecal samples. However, the present study showed that the *ail* -gene by PCR test does not guarantee detection of pathogenic strains. The discovery of multiresistant *Y. enterocolitica* in Finnish patients was a further warning of the global increase in antibiotic resistance in all bacteria. *Y. enterocolitica* strains with antimicrobial resistance associated significantly with travelling abroad. For future molecular surveillance and detection of outbreaks caused by *Y. enterocolitica*, MLVA is a sensitive and repeatable method.

Keywords: case-control study, foodborne pathogen, epidemiology, molecular typing, *Yersiniae, Yersinia enterocolitica*

Tiivistelmä

Leila M Sihvonen. Clinical Isolates of *Yersinia enterocolitica* in Finland - Identification and Epidemiology [Potilaista eristetyt *Yersinia enterocolitica* –bakteerikannat Suomessa - tunnistus ja epidemiologia]. Terveyden ja hyvinvoinnin laitos (THL). Tutkimus 117. 145 sivua. Helsinki, 2014.
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Yersinia enterocolitica on zoonoottinen, eli eläinten ja ihmisten välillä tarttuva bakteeri. Se aiheuttaa ihmiselle yersinioosin, joka ilmenee tyypillisimmin suolistotulehduksena. Yleisemmin yersinioosi tarttuu elintarvikevälitteisesti. Suolistozoonoosin aiheuttajana se on raportoitu kolmanneksi yleisimmäksi bakteeriksi kampylobakteerin ja salmonellan jälkeen sekä Suomessa, että koko Euroopassa. Osa *Yersinia enterocolitica* –infektioista on oireiltaan lieviä, mutta joskus esiintyy myös vakavampia taudinkuvia erityisesti riskiryhmiin kuuluvilla henkilöillä. Lisäksi yersiniainfektio voi aiheuttaa monia jälkitauteja, muunmuassa reaktiivista niveltulehdusta, joka vaikeimmillaan voi muuttua krooniseksi.

Y. enterocolitica on heterogeeninen bakteeri, jonka kannat voivat olla invasiivisia (patogeeniset biotyypit joilla on virulenssiplasmidi, pYV) tai mahdollisesti jopa kokonaan harmittomia (biotyyppi 1A). *Y. enterocolitica* on elintarvikevälitteisten epidemioiden aiheuttaja, jonka yksi erityispiirre on, että se pystyy lisääntymään jääkaappilämpötiloissa. Sen tunnistaminen laboratoriossa on kuitenkin haasteellista, sillä sen voi helposti sekoittaa sukulaislajeihin, ns. ympäristöyersinioihin eli *Y. enterocolitican* -kaltaisiin lajeihin, joita pidetään ihmisillä pääosin harmittomina. Lisäksi se hidaskasvuisena voi välillä jäädä kokonaan löytämättä näytteistä. Euroopan tasolla kirjavat tunnistus– ja ilmoituskäytännöt aiheuttavat bakteerin diagnosoitiikassa suuria vaihteluita eri maiden välillä.

Tämän tutkimuksen tarkoituksena oli selvittää suomalaisista potilasnäytteistä eristettyjen *Y. enterocolitica* kantojen alatyyppien kirjoa, herkkyyttä mikrobilääkkeille ja selvittää millä menetelmillä kannat parhaiten löytyvät potilasnäytteistä, sekä parantaa epidemianselvityksessä käytettäviä tyypitysmenetelmiä. Lisäksi selvitettiin yersiniainfektioon sairastuneiden potilaiden kliinistä taudinkuvaa, jälkitautien esiintyvyyttä sekä infektion lähteitä. Materiaaliksi työtä varten kerättiin vuoden 2006 aikana kymmenestä suomalaisesta kliinisen mikrobiologian laboratoriosta eristetyt *Yersinia* -bakteerit taustatietoineen. Kantoja tutkitiin erilaisten ilmiasuun (fenotyyppiin) ja perimään (genotyyppiin) perustuvin menetelmin. Potilaille, joista kannat oli eristetty, tehtiin lisäksi tapaus-verrokkitutkimus.

Patogeeniset, virulenssiplasmidin omaavat (pYV+) *Y. enterocolitica* kannat oli mahdollista erottaa fenotyyppiin perustuvilla testeillä ja kylmärikastus paransi kantojen

löytymistä näytteistä. Epidemianselvityksen työkaluksi näille kannoille kehitettiin DNA:n toistojaksojen eroihin perustuva “Multilocus variable tandem repeats analysis” eli MLVA -tyypitysmenetelmä. MLVA osoittautui ylivoimaiseksi erottelukyvyltään aiemmin käytettyyn pulssikenttäelektroforeesiin (PFGE) verrattuna. Sen sijaan *Y. enterocolitica* biotyypin 1A kantojen erottaminen ympäristöyersinioista oli vaikeaa fenotyyppisten testien avulla. Geneettinen tyypitys MLST-menetelmällä osoitti biotyyppi 1A kantojen jakautuvan kahteen geneettisesti täysin erilliseen ryhmään. Lisäksi tutkimuksessa löydettiin ensi kerran virulenssigeeni *ail Y. enterocolitica* biotyyppi 1A kannalta. *Y. enterocolitica* biotyyppi 1A:n kantojen aiheuttamat oireet erosivat pYV+ kantojen aiheuttamista oireista. Potilaille tehdyssä kyselytutkimuksessa yleisimmäksi *Y. enterocolitica* –bakteerin patogeenisten biotyyppien tartunnan riskitekijäksi nousi huonosti kypsennetty tai raaka sianliha. Tartunnan saaneista potilaista 23% oli matkustanut ulkomailla ennen sairastumistaan. *Y. enterocolitica* pYV+ kannoista 19% oli kehittänyt vastustuskyvyn neljälle tai useammalle testatulle mikrobilääkkeeelle ja resistenssi korreloi vahvasti ulkomailta saatuun tartuntaan. Alle 3-vuotiaat lapset olivat yliedustettuina patogeenisten biotyyppien infektioissa.

Ihmisten ulostenäytteitä tutkittaessa kylmärikastus, kasvatus CIN-maljalla ja virulenssiplasmidin osoitaminen, olivat tehokkaita patogeenisten *Y. enterocolitica* kantojen eristämisessä. Fenotyyppiset testit ja virulenssiplasmidin testaus erottivat patogeeniset *Y. enterocolitica* -kannat BT 1A -kannoista ja muista yersinioista. Tutkimus kuitenkin osoitti, että kromosomaalisen *ail* -geenin löytyminen PCR-testissä ei täysin takaa sitä, että kyseessä olisi *Y. enterocolitican* klassisesti patogeeninen kanta. Mikrobilääkkeille reisistenttien *Y. enterocolitica* -kantojen löytyminen suomalaisista potilaista oli jälleen yksi varoitus maailmanlaajuisesta antibioottiresistenssin lisääntymisestä kaikilla bakteereilla. *Y. enterocolitica* bakteerin resistentit kannat liittyivät merkitsevästi ulkomailla *matkustamiseen. Y. enterocolitica* bakteerin aiheuttamien epidemioiden osoittamiseen sekä tartuntareittien selvittämiseen MLVA –menetelmä on soveltuva hyvän erottelukykynsä sekä toistettavuutensa ansiosta.

Avainsanat: elintarvikevälitteinen patogeeni, epidemiologia, molekyylityypitys, tapaus-verrokki tutkimus, *Yersiniae*, *Yersinia enterocolitica*

Sisällys

List of original papers

This thesis is based on the following original articles, which are referred in the text by their Roman numerals (I-V). In addition, some unpublished data are presented.

I Sihvonen, L.M., K. Haukka, M. Kuusi, M.J. Virtanen, A. Siitonen, YE study group. 2009. *Yersinia enterocolitica* and *Y. enterocolitica*-like species in clinical stool specimens of humans: identification and prevalence of bio/serotypes in Finland. Eur J Clin Microbiol Infect Dis. 7:757-65.

II Huovinen, E., L.M. Sihvonen, M. J. Virtanen, K. Haukka, A. Siitonen, M. Kuusi. 2010. Symptoms and sources of *Y. enterocolitica* infections in Finland: a case-control study, BMC Infect Dis 10:122.

III Sihvonen, L.M., S. Toivonen, K. Haukka, M. Kuusi, M. Skurnik, A. Siitonen. 2011. Multilocus variable-number tandem-repeat analysis, pulsed-field electrophoresis, and antibiotic susceptibility patterns in differentiation of sporadic and outbreak-related strains of *Yersinia enterocolitica*. BMC Microbiol 11:42.

IV Sihvonen, L.M., S. Hallanvuo, K. Haukka, M. Skurnik, A. Siitonen. 2011. The *ail* gene is present in some *Yersinia enterocolitica* biotype 1A strains. Foodborne Pathog Dis 8:455.

V Sihvonen, L.M., K. Jalkanen, E. Huovinen, S. Toivonen, J. Corander, M. Kuusi, M. Skurnik, A. Siitonen, K. Haukka. 2012. Clinical isolates of *Yersinia enterocolitica* Biotype 1A represent two phylogenetic lineages with differing pathogenicity-related properties. BMC Microbiol 12:208.

The authors´s contribution

Study I

Participated in design of the study, collecting the bacterial strains and their identification, collecting and analysis of the data and wrote the manuscript.

Study II

Participated in design of the study, collecting the bacterial strains and their identification, and writing the manuscript.

Study III

Participated in the design of the study, did or supervised the MLVA, PFGE, DNA sequencing, and antimicrobial susceptibility testing, carried out the data analysis, and wrote the manuscript.

Study IV

Participated in the design of the study, did DNA sequencing, and wrote the manuscript.

Study V

Participated in the design of the study, did or supervised the MLST, 16 rRNA sequencing, *ystA* and *ystB* PCRs, carried out the data analysis and wrote the manuscript.

Abbreviations

ail	gene encoding attachment and invasion locus
adk	gene encoding adenylate kinase
a*rgA*	gene encoding N-acetylglutamate synthase
aroA	gene encoding 3-phosphoshikimate-1-carboxylvinyltransferase
BAPS	Bayesian Analysis of Population Structure
BT	Biotype
CIN	Cefsulodin-irgasan-novobiocin agar
CR-MOX	Congo-red magnesium-oxalate agar
ECDC	European Centre for Disease Control
EFSA	European Food Safety Authority
glnA	gene encoding glutamine synthase
gyrA	gene encoding DNA gyrase subunit A
gyrB	gene encoding DNA gyrase subunit B
HLA B27	Human leukocyte antigen B27
Inv	Invasin
lcrE	Gene encoding low-calcium response region gene E
LPS	Lipopolysaccharide
MALDI-TOF	Matrix-assisted laser desorption/ionization-time of flight mass spectrometry
MLST	Multilocus sequence typing
MLVA	Multilocus variable number tandem-repeat analysis
myf	Mucoid yersinia fimbrillae
NCTC	National Collection of Type Cultures
NIDR	Finnish National Infectious Diseases Register
NTB	Non-typeable biotype
PCR	Polymerase chain reaction
PFGE	Pulsed-field gel electrophoresis
pYV	Plasmid for *Yersinia* virulence
ReA	Reactive arthritis
ST	Serotype
thrA	gene encoding aspartokinase I/homoserine dehydrogenase I
trpE	gene encoding anthranilate synthase component I
virF	Virulence regulon transcriptional activator
YE	*Yersinia enterocolitica*
Yst	Yersinia heat-stable toxin
Yop	Yersinia outer protein
ystA	gene encoding *Yersinia* heat-stable toxin A
ystB	gene encoding *Yersinia* heat-stable toxin B
YadA	Yersinia adhesin A

1 Introduction

Y. enterocolitica is an enteropathogenic bacteria causing zoonotic gastroenteritis in humans. Commonly, sequelae such as reactive arthritis appear after gastroenteritis. *Y. enterocolitica* is a heterogenic and potentially pathogenic species (Bottone 1999). There also exist closely related bacterial species, the "*Y. enterocolitica* –like" species that resemble *Y. enterocolitica* in phenotypic tests. The significance of *Yersinia* in clinical samples can be difficult to deduce, particularly if biotype (BT) and serotype (ST) information is lacking.

By early 2000 the number of clinical *Y. enterocolitica* findings reported in the Finnish National Infectious Diseases Register (NIDR) was increasing compared to salmonellosis. The incidence of *Yersinia* was more than 1.5 times higher than domestic *Salmonella* by early 2000. In contrast to most European countries, *Yersinia* was a more significant cause of domestic zoonosis than Salmonella in Finland. At the European level, the number of *Yersinia* incidence in Finland have been among the highest in EU, varying annually from 500-700 cases - approximately 10-11 per 100 000 population (EFSA 2005; EFSA 2006; EFSA 2013).

Records in the Finish NIDR before 2010 lacked information pertaining to the BTs of *Y. enterocolitica*. Reports of *Yersinia* in Finland are made directly by routine laboratories and the strains were only seldom (mainly only if outbreak was suspected or there were difficulties in identification) sent to National reference laboratory. Therefore, little information about the virulence, BT/STs and susceptibility to antimicrobials of *Y. enterocolitica* strains existed at the national level. Further, clinical samples frequently contain non-pathogenic *Y. enterocolitica* BT 1A strains. However, no genotypic data or prevalence of Finnish BT 1A strains was available.

The purpose of this thesis was to characterise clinical *Y. enterocolitica* and *Y. enterocolitica*-like strains isolated in Finland with phenotypic and genotypic methods so as to gain insight into the occurrence of different subtypes, and virulence-associated markers. Furthermore, the study aims to evaluate methods for routine laboratories for the identification of pathogenic *Y. enterocolitica* strains, as well as methods for epidemiological outbreak investigations. In addition, the sources and symptoms of *Y. enterocolitica* infections were investigated in a case-control study.

2 Review of the Literature

2.1 The genus *Yersinia*

The genus *Yersinia* within the class gamma proteobacteria, family *Enterobacteriaceae,* currently comprises currently 17 validly named species. Three species, *Y. pestis, Y. pseudotuberculosis* and *Y. enterocolitica,* are pathogenic to mammals, and one, *Y. ruckerii,* to fish (Ewing *et al.* 1978). Other species of the genus are considered environmental strains since they appear mainly in freshwater aquatic and terrestial ecosystems (Robbins-Browne 2007). These species are *Y. bercovieri, Y frederiksenii, Y. intermedia, Y. kristensenii, Y. mollaretii, Y. rohdei, Y. aldovae, Y. alecksiciae* (Sprague and Neubauer 2005), *Y. similis* (Sprague *et al.* 2008), *Y. massiliensis* (Merhej *et al.* 2008), *Y. entomophaga* (Hurst *et al.* 2010), *Y. pekkanenii* (Murros-Kontiainen *et al.* 2010) and *Y. nurmii* (Murros-Kontiainen *et al.* 2010).

2.1.1 *Yersinia enterocolitica*

Y. enterocolitica is the most common pathogenic *Yersinia* infecting humans. Strains of *Y. enterocolitica* strains are classified into six BTs 1A, 1B, 2, 3, 4, and 5 based on the ability to metabolize certain selected substrates as shown in Table 1 (Wauters *et al.* 1987; Wauters *et al.* 1988). At least 57 different serotypes have been identified in *Y. enterocolitica* strains (Table 2) (Robbins-Browne 2007). Based on DNA-DNA reassociation values and differences in16S rRNA *Y. enterocolitica* has been divided into two subspecies: ssp. *enterocolitica* and ssp. *palearctica* (Neubauer *et al.* 2000). Ssp. *enterocolitica* is comprised of BT 1B strains and ssp. *paleartica* includes the strains of the BTs 1A and 2-5. Based on differences in whole genomes by microarray analysis appearance of a third subspecies, which should constitute BT 1A strains, has been suggested (Howard *et al.* 2006). In whole genome analysis of 100 *Y. enterocolitica* strains belonging to different BTs it was shown that BT 1B and BT 1A are more closely relate to each other than to other BTs, and BTs 2-5 are very closely related (Reuter *et al.* 2012).

2.1.2 *Yersinia enterocolitica* -like species

Species related to *Y. enterocolitica* are sometimes called *Yersinia enterocolitica* -like bacteria. This refers to the *Yersinia* species that are easily misidentified as *Y. enterocolitica* in traditional phenotypic tests. In the present study *Y. bercovieri, Y. mollaretii, Y. intermedia, Y. kristensenii, Y. frederiksenii, Y. aldovae, Y. alecksiciae, Y. massiliensis* and *Y. rohdei* are referred to *Y. enterocolitica* –like species. The *Y. enterocolitica* –like species, also called environmental *Yersinia,* are common in environmental and animal sources (Bottone 1999)

Table 1. Classification of *Y. enterocolitica* BTs[1] based on the biochemical reactions[2] of strains.

Test	1A	1B	2	3	4	5
Esculin hydrolysis	D	-	-	-	-	-
Salicin (acid production)	+	-	-	-	-	-
ß-D-glucosidase	+	-	-	-	-	-
Pyratzinamidase	+	-	-	-	-	-
Lipase (Tween hydrolysis)	+	+	-	-	-	-
Indole production	+	+	(+)	-	-	-
D-xylose (acid production)	+	+	+	+	-	D
Trehalose	+	+	+	+	+	-
Voges-Proskauer reaction	+	+	+	+	+	(+)

Adapted from Wauters *et al* 1987.

[1] Modified from references

[2] +, positive; -, negative; (+) ,delayed reaction,; D, different reactions.

Table 2. Serotypes associated with different BTs of *Y. enterocolitica* and *Y. enterocolitica* –like species (Robins-Browne 2007).

	Serotype(s)
Y. enterocolitica	
1A	O:4; O:5; O:6,30; O:6,31; O:7,8; O:7,13; O:10; O:14; O:16; O:21; O:22; O:25; O:37; O:41,42; O:46; O:47; O:57; NT[1]
1B	O:4,32; O:8; O:13[a]; O:13b; O:16; O:18; O:20; O:21; O:25; O:41,42; NT
2	O:5,27; O:9; O:27
3	O:1,2,3; O:3; O:5,27
4	O:3
5	O:2,3
Y. bercovieri	O:8; O:10; O:58,16
Y. frederiksenii	O:3; O:16; O:35; O:38; O:44
Y. intermedia	O:17; O:12;46; O:35; O:37; O:40; O:48; O:52; O:55
Y. kristensenii	O:11; O:12,25; O: 12,26; O:16; O:16,29; O:28,50; O:46; O:52; O:59; O:61
Y. mollaretii	O:3; O:6,30; O:7,13; O:59; O:62,22

[1] NT, not typeable

2.2 Virulence of *Y. enterocolitica*

The virulence of *Y. enterocolitica* strains has been shown to vary in the mouse model: BT 1B *i.e.* ssp. *enterocolitica* has highly-virulent strains, which are lethal to

mice in low doses, whereas BTs 2-5 are less pathogenic (Carter 1975). BT 1A strains are regarded as non-virulent since they lack most of the classical virulence associated genetic markers of *Y. enterocolitica*, however the role of BT 1A is controversial. All *Y. enterocolitica* BTs are highly acid resistant which is mediated by the ability to produce urease (de Koning-Ward and Robins-Browne 1995; Tennant *et al.* 2008). This helps *Yersinia* strains to overcome the gastric acid barrier of stomach before entering the small intestine. All *Y. enterocolitica* strains have flagella, which allow movement in a desired location (Aleksic and Bockemuhl 1987; Wauters *et al.* 1991).

2.2.1 Virulence associated markers of BTs 1B and 2-5

To express the full potential virulence of *Y. enterocolitica* BTs 1B and 2-5, a plasmid for *Yersinia* virulence (pYV) encoding approximately 50 proteins is needed (Gemski *et al.* 1980; Zink *et al.* 1980; Portnoy *et al.* 1981). The most important virulence factors encoded by pYV are a type III secretion system and effectors known as *Yersinia* outer proteins (yops). (Cornelis and Wolf-Watz 1997). Yops enable survival and growth of *Yersinia* inside the host's cells. Multifunctional Yersinia adhesion A protein (YadA) mediates attachment to mucus and epithelial cells, is also pYV -encoded (Paerregaard *et al.* 1991). In addition, YadA plays an important role in complement resistance and immune evasion (Galindo *et al.* 2011).

pYV alone is not enough to confer virulence, chromosomal factors are also essential (Heesemann and Laufs 1983; Heesemann *et al.* 1984). Chromosomal locus *inv* encodes invasion protein invasin, which binds to B1 integrins and facilitates penetration of host cells (Pepe and Miller 1993; Pepe and Miller 1993). Chromosomal encoded attachment and invasion locus (*ail*) encodes proteins required for invasion, attachment and serum resistance (Miller *et al.* 1989; Miller *et al.* 2001). Some *Yersinia* produce mucoid yersinia fimbrillae (Myf) associated with the colonization of intestinal epithelium (Iriarte and Cornelis 1995). *Y. enterocolitica* heat-stable enterotoxins (YST) induce diarrhoea (Delor and Cornelis 1992). *Hre* (host responsive element) genes encoded proteins involved in the stress response, iron starvation, cell envelope maintenance, transcription regulation, and other unknown functions (Gort and Miller 2000). Homologous insecticidal toxin gene clusters have been described from 4/O:3 and 2/O:9 strains and eventhought they are only expressed at low temperatures (Bresolin *et al.* 2006), they are thought to contribute to virulence (Tennant *et al.* 2005; Bresolin *et al.* 2006; Batzilla *et al.* 2011). Lipopolysaccharide (LPS) found on the outer membrane of Gram-negative bacteria is implicated as a virulence factor due to its lipid A part of the molecule, which is a endotoxin. In addition, the O-side chains of LPS of *Y. enterocolitica* 4/O:3 and 1B/O:8 have shown to contribute to the virulence (Skurnik and Toivanen 1993; Zhang *et al.* 1997; Skurnik *et al.* 1999; Najdenski *et al.* 2003).

Futhermore, *Y. enterocolitica* BT 1B strains have a chromosomally encoded 35 – 45 kb pathogenicity island (HPI) encoding genes involved in yersiniabactin-mediated iron uptake (Pelludat *et al.* 1998). *Y. enterocolitica* BT 1B also have *yts1* type II and *ysa* type III secretion systems which enhanced virulence (Haller *et al.* 2000; Iwobi *et al.* 2003). The major virulence factors of *Y. enterocolitica* are listed in the Table 3.

2.2.2 Virulence associated markers of *Y. enterocolitica* BT 1A

While the strains of BTs 1B and 2-5 have well-characterized clinical significance, the strains of BT 1A are controversial as pathogens. *Y. enterocolitica* BT 1A strains lack pYV and most of the chromosomal virulence-associated determinants. The choromosomally encoded *ail* –gene has been assumed to be absent from *Y. enterocolitica* BT 1A strains (Miller *et al.* 1989) and the gene *inv* for invasion has been shown to be non-functional in most strains (Pierson and Falkow 1990). Tested BT 1A strains have also been avirulent in the mouse model (Carter 1975).

Nevertheless, *Y. enterocolitica* BT 1A produce urease and have a variety of different O-side chains of LPS, among those O:5 and O:8 are shared with pathogenic BTs. Most of the strains of *Y. enterocolitica* BT 1A possess genes encoding heat-stable enterotoxin YstB, structurally and functionally homologous to the heat-stable enterotoxins of enterotoxigenic *E. coli* (ETEC) and *Vibrio cholera* non-01 (Granum 2006). The enterotoxins cause diarrrhoea by stimulating cGMP synthesis in the intestinal brush border, leading to an overall effect of fluid loss. Some BT 1A strains have been reported of being capable to invade epithelial cells in vitro (Robins-Browne 1989; Grant *et al.* 1999) and multiply inside of macrophages (McNally *et al.* 2006).

2.2.3 Virulence associated markers of *Y. enterocolitica* –like species

Y. enterocolitica –like species lack pYV and most of the chromosomal virulence associated markers. However, there are reports of heat-stable enterotoxins associated with some strains of *Y. enterocolitica* –like species (Delor *et al.* 1990; Sulakvelidze *et al.* 1999). They are frequently isolated from healthy individuals, as well as symptomatic patients, but their role to disease is as controversial as the *Y. enterocolitica* BT 1A strains (Loftus *et al.* 2002).

Table 3. Major virulence factors of *Y. enterocolitica.*

Virulence factor	Function	BT 1B	BTs 2-5	BT 1A	Reference
pYV					
Ysc T3SS and Yops	Counteract host immune defences and disrupt host signalling	+	+	-	(Cornelis and Wolf-Watz 1997; Viboud and Bliska 2005)
***Yersinia* adhesion (YadA)**	Mediates adherence to cells and extracellular matrix and complement evasion	+	+	-	(El Tahir and Skurnik 2001)
Chromosomal					
Flagella	Motility in initiating host cell invasion.	+	+	+	(Young *et al.* 2000)
Lipopolysaccharide (LPS)	Endotoxin, complement evasion	+	+	+	(Kanamori 1976; Iriarte and Cornelis 1995; Skurnik 2003)
Urease	Acid resistance	+	+	+	(de Koning-Ward *et al.* 1995)
Invasin (Inv)	Facilitates penetration to host cells	+	+	+[1]	(Miller and Falkow 1988)
Attachment Invasion locus (Ail)	Attachment and invasion factor, complement evasion	+	+	-/+[2]	(Miller *et al.* 1989)
Mucoid Yersinia fimbrillae (Myf)	Involves colonising intestinal epithelium	+	+	+	(Iriarte and Cornelis 1995)
Host responsive element P (HreP)	Subtilisin/Kexin-Like Protease	+	+	+	(Gort and Miller 2000; Heusipp *et al.* 2001)
Heat-stable enterotoxins (YST)	Fluid accumulation in the intestine inducing diarrhoea	+	+	+	(Delor and Cornelis 1992)
Insecticidal Toxin Complex (TC)	Possible role in persistence of bacteria in the gastrointestinal track	+	+	+	(Tennant *et al.* 2005)
High-pathogenicity Island (HPI)	Encodes yersinibactin responsible for iron uptake	+	-	-	(Carniel 1999)

[1] Mostly unfunctional, [2] Found only seldom

2.3 Clinical manifestations caused by *Y. enterocolitica*

Y. enterocolitica is an enteric pathogen that usually causes self-limiting gastroenteritis and capable of causing a wide variety of other clinical disorders in humans.

2.3.1 Acute infections

Gastroenteritis caused by *Y. enterocolitica* typically has the symptoms of diarrhoea, abdominal pain, fever and vomiting. These symptoms appear after 1-11 days' incubation period and last about 5-14 days (Cover and Aber 1989). Occasionally, symptoms may last longer, sometimes even several months (Cover and Aber, 1989). *Y. enterocolitica* has an infectious dose of 10^8 -10^9 organisms (De Berardis *et al.* 2004), but the infectious dose may be lower for individuals with gastric hypoacidity (Foberg *et al.* 1986; de Koning-Ward and Robins-Browne 1995). *Y. enterocolitica* more frequently affects children than adults (Cohen 1991; Lee *et al.* 1991; Helms *et al.* 2006; Boqvist *et al.* 2008; Zheng *et al.* 2008; Rosner *et al.* 2010). This may be due to their immature and relatively unchallenged immune system (Koehler *et al.* 2006; Boqvist *et al.* 2008).

Gastroenteritis normally heals spontaneously, but sometimes complications appear, especially with immune-compromised patients. *Y. enterocolitica* may cause an invasive disease such as mesenteric lymphadenitis, terminal ileitis mimicking appendicitis (Matsumoto *et al.* 1991; Tuohy *et al.* 1999; Perdikogianni *et al.* 2006; Antonopoulos *et al.* 2008), septicemia (Cornelis *et al.* 1987) or pharyngitis (Tacket *et al.* 1983). *Y. enterocolitica* has been a significant cause of life-threating blood transfusion associated bacteremia (Bottone 1999; Benavides *et al.* 2003; Brecher and Hay 2005; Leclercq *et al.* 2005; Guinet *et al.* 2011). Around 46% of the documented cases of clinical sepsis due to contaminated red blood cell preparations have been caused by *Y. enterocolitica* (Wagner 2004). In rare cases *Y. enterocolitica* can also cause primary cutaneous infection such as abscesses (Gumaste *et al.* 2012). *Y. enterocolitica* related pneumonia has also been reported (Wong *et al.* 2013). Further, the association of *Y. enterocolitica* with gastrointestinal disorders such as inflammatory bowel disease (IBD), Chron's disease, non-steroidal anti-inflammatory drugs (NSAID) induced colitis and collagenous colitis, has been addressed in several studies (Mäkinen *et al.* 1998; Bohr *et al.* 2002; Saebo *et al.* 2005; Knösel *et al.* 2009).

The clinical relevance of *Y. enterocolitica* BT 1A strains in is controversial. In many studies BT 1A is considered totally harmless to humans (Bottone 1999). However, some studies have reported that the clinical picture of patients with BT 1A infection

has been shown to be indistinguishable to those with a classical pathogenic isolate (Noble *et al.* 1987; Burnens *et al.* 1996).

2.3.2 Extra intestinal complications

Y. enterocolitica sometimes causes extra intestinal complication, which usually are post-infectious and appear typically 7-30 days after the acute disease (McDowell and McElvaine 1997). Extra intestinal manifestations denote disorders such as reactive arthritis (ReA), erythema nodosum (Hoogkamp-Korstanje and Stolk-Engelaar 1995), uveitis, glomerulonehritis, carditis, thyroiditis (Bottone 1999) and ankylosing spondylitis (Hrycaj and Lacki 2003).

ReA is the most common post-infectious complication of *Y. enterocolitica*. The term reactive arthritis was first used in late 60´s to describe arthritis that develops soon after or during the infection elsewhere in the body, but where the micro-organism cannot be recovered from the joint (Ahvonen *et al.* 1969). However, later it has been discovered that a ReA triggering microbe can persist in the host and antigens of the *Y. enterocolitica* (Granfors *et al.* 1989) and other ReA causing organisms have be detected in synovial fluid or tissue. The clinical picture of ReA is characterized by asymmetrical oligoarthritis *i.e.* with an uneven distribution, typically affecting unpaired large joints of the lower limbs joints (Petersel and Sigal 2005). In about 25% of the patients with ReA, the preceding infection has been asymptomatic (Hannu *et al.* 2006). A significant percentage of the diseased people suffer from persistent or relapsing illnesses (McDowell and McElvaine 1997).

The human leukocyte antigen HLA-B27 has been strongly associated with sondyloarthropathies, such as ReA, after *Yersinia* infection, although the mechanism has remained elusive (Bowness 2002). Since HLA-B27 is common in many human populations, it has been assumed that it has some selective advantages (Khan 1995). HLA-B27 is not required for the development of ReA, however, its presence is contributing to the chronicity of the disease (Leirisalo-Repo 2005). The mechanism of interaction between the pathogen causing ReA and the host is poorly understood. The interaction of *Yersinia* adhesion A (YadA) protein with collagen has been proposed to contribute to the development of ReA (Laitenen *et al.* 1972; Schmid *et al.* 2004; Eitel *et al.* 2005). ReA disease has also been reported to be associated with BT 1A (Ebringer *et al.* 1982; Skurnik *et al.* 1983). In addition, antigens IgG, IgA and IgM antibodies against a strain of BT 1A/O:6 has been detected in a patient with ReA (Skurnik *et al.* 1983).

2.4 Epidemiology of *Y. enterocolitica* infections

Y. enterocolitica is common in cold or temperate climate zones since it is well adapted to grow in low temperatures (Palonen *et al.* 2010). However, it can also be

found in subtropical or tropical latitudes all over the world (Ding *et al.* 1986; Falcao *et al.* 2006; Adjei *et al.* 2009; Hanifian and Khani 2012). In Europe *Y. enterocolitica* is the third most commonly, in Finland the third commonly reported, foodborne bacterial pathogen after *Campylobacter* and *Salmonella* (EFSA 2013). The most common pathogenic bio/serotype of *Y. enterocolitica* is 4/O:3 world wide. Another frequently appearing pathogenic bio/serotype is 2/O:9. The highly virulent bio/serotype 1B/O:8 are sometimes called as "New world strains" since they are commonly in North American (Wren 2003). Today, however, the BT/ST 1B/O:8 are reported seldom.

The Finish NIDR consists of mandatory laboratory reporting of diagnostic findings for microbiologically confirmed infectious diseases. In Finland, when a bacterial cause of diarrhoea is investigated using stool culturing the presence of *Salmonella*, *Shigella*, *Campylobacter* and *Yersinia* are tested. Laboratory confirmed cases of *Yersinia* are reported into the NIDR. The number of culture or antibody confirmed *Y. enterocolitica* cases in Finland has been around 400-600 annually - an incidence rate of 10 -11 per 100 000 people per year (Figure 1).

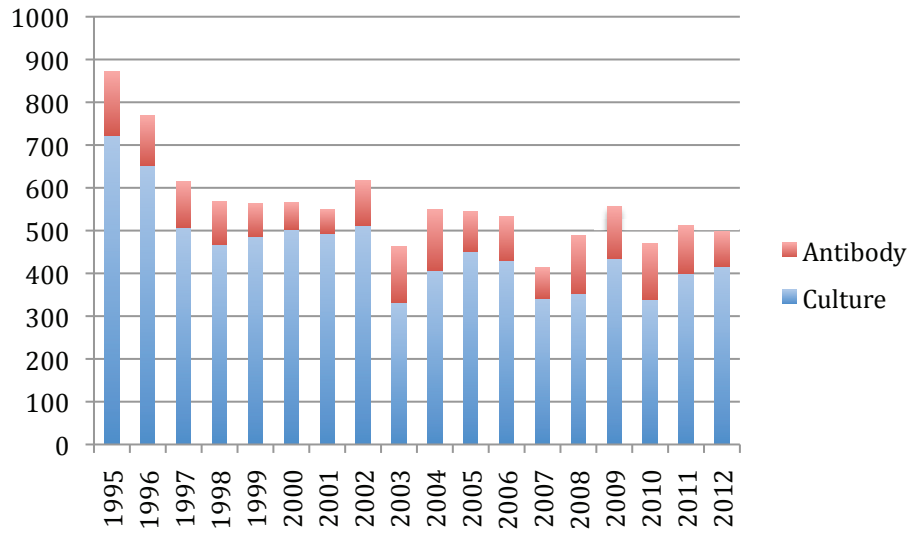

Figure 1. *Y. enterocolitica* infections registered in the National Infectious Diseases Register (NIDR) in Finland in 1995-2012.

At the European level, prevalence of confirmed cases of yersiniosis in 24 EU member states in 2011, varied from 0 - 11.4 cases per 100 000 (EFSA 2013). Of the confirmed yersiniosis cases with known hospitalisation (Austria, Estonia, Hungary,

Irland, Latvia, Lithuania, Luxenburg, Romania, Slovenia), 55.2% were hospitalised in 2011 (EFSA 2013). Nevertheless, there has been a decreasing trend in confirmed cases of human yersiniosis in the EU from 2007-2012 (EFSA 2013). Because of the differences in diagnostics and reporting systems, it is not possible to compare the prevalence between countries. Therefore, the number of cases is likely under-estimated.

2.4.1 Role of animals as reservoirs of *Y. enterocolitica*

Raw pork meat has been shown to be the most important reservoir for human pathogenic *Y. enterocolitica* (Tauxe *et al.* 1987; Fredriksson-Ahomaa *et al.* 2006; Boqvist *et al.* 2008; Rosner *et al.* 2012) Also, butchers, who handled swine throats and intestines had elevated levels (27%) of *Y. enterocolitica* O:3 antibodies compared to blood donors (10%) (Merilahti-Palo *et al.* 1991). Contact with birds and eating take-away food has also been recognised as a risk factor for *Yersinia* infection (Rosner *et al.* 2012).

Systematic reports of the prevalence of *Y. enterocolitica* in animals and food, however, are limited available (Fredriksson-Ahomaa *et al.* 2007). In 2011 only 9 and 11 member states of the EU reported *Yersinia* findings in food and animals, respectively (EFSA 2013). BTs 2-5 can be isolated from animals, most commonly from swine. Strains of bio/serotype 4/O:3 are frequently isolated from pigs (Fredriksson-Ahomaa *et al.* 2001; Korte *et al.* 2004; Fredriksson-Ahomaa *et al.* 2007; Wesley *et al.* 2008). Also cattle, sheep, birds, rodents, and pet animals can carry pathogenic *Y. enterocolitica*. Clinical disease, caused by *Y. enterocolitica* in animals is not very common, however (Bottone 1999).

Y. enterocolitica BT 1A strains are common isolates from aquatic and terrestrial environments, but have also been isolated from milk products, pork and beef (Falcao *et al.* 2006). In a British study BT 1A strains were found in 4.5% of the studied cattle, 5% of the sheep and 5% of pigs at slaughter (Milnes *et al.* 2008). In a recent study in Sweden, prevalence of *Y. enterocolitica* BT 1A in sheep was found to be 35% (Soderqvist *et al.* 2012). Also the goats have been recognised as a reservoir of BT 1A (Lanada *et al.* 2005; Arnold *et al.* 2006). Furthermore, other domestic animals, as well as rodents, birds, frogs, fish, fleas are recognised as potential reservoirs of *Y. enterocolitica* (Kapperud 1991; Bottone 1997).

In contrast to other enteropathogens such as *Campylobacter, Y. enterocolitica* strains cannot be grouped into human and animal strains (Reuter et al. 2012). Therefore, there have not been detected subsets of animal isolates of pathogenic *Y. enterocolitica* that would be more likely to cause disease to humans (Reuter et al. 2012).

2.4.2 Outbreaks

Y. enterocolitica cases are mostly sporadic and outbreaks are not quite common. However, outbreaks in different parts of world are reported almost annually. Often the cause of the outbreak cannot be confirmed. Some published outbreaks of *Y. enterocolitica* are listed in the Table 4.

Table 4. Outbreaks of *Y. enterocolitica* published in scientific journals or in reports of public health authorities.

Year	Country	City/State	Location	Bio/serotype	Suorce/Vector	Cases	Reference
1971	Czechoslovakia		Nursery	4/O:3	nd[a]	15	(Olsovsky *et al.* 1975)
1972	Finland		Hospital	O:9	Hospital patient	7	(Toivanen *et al.* 1973)
1973	Finland	Kirkkonummi	Garrison	O:9; O:3	nd	117	(Lindholm and Visakorpi 1991)
1973	USA	NC		1B/O:8	Dog	16	(Gutman *et al.* 1973)
1976	USA	NY		1B/O:8	Chocolate milk	36	(Black *et al.* 1978)
1976	Canada	Montreal		O:5,27	Non-pasteurized milk[c]	138	(deGrace *et al.* 1976; Kasatiya 1976)
1976-77	Israel		Kibbutz	4/O:3; 3/O:1,2,3	nd	5	(Shmilovitz and Kretzer 1978)
1980	Japan	Okinawa	School	4/O:3	Milk	1051	(Maruyama 1987)
1980	Canada		Hospital	1A/O:5	Person-to-person	9	(Ratnam *et al.* 1982)
1981	Canada	SK	Household	1B/O:21[a]	nd	3	(Martin *et al.* 1982)
1981	USA	NY	Summer camp	1B/O:8	Food handler	239	(Shayegani *et al.* 1983; Morse *et al.* 1984)
1981-82	USA	WA		1B/O:8	Tofu	87	(Tacket *et al.* 1985)
1982	USA	TN, AR, MS		O:13a; O:13b	Pasteurized milk[c]	172	(Tacket *et al.* 1984; Toma *et al.* 1984)
1982	Finland	Espoo	Cafeteria	O:3	nd	26	(Tuori and Valtonen 1983)
1983	Hungary			O:3	Brawn	8	(Marjai *et al.* 1987)
1984	UK			1A/ O:6,30	Pasteurized milk	2	(Barrett 1986)
1984	Canada			4/O:3	Well water	3	(Thompson and Gravel 1986)
1985	UK			1A/O:10	Pasteurized milk	19	(Greenwood and Hooper 1990)
1985	UK			1A/O:6,30	Pasteurized milk	17	(Greenwood and Hooper 1990)
1986	Belgium		Nursery	4/O:3	nd	21	(Van Ossel and Wauters 1990)

1986	UK		Hospital	1A/O:6,30	Patient		(McIntyre and Nnochiri 1986)
1987	USA		Family	nd	nd	2	(Rose *et al.* 1987)
1987-88	Australia			O:3; BT1A/O:6:30	nd[c]	11	(Butt *et al.* 1991)
1988	USA	Georgia		O:3; O:1,2,3	Chitterlings	15	(Lee *et al.* 1990)
1988	Sweden	Bjärred peninsula		O:3	milk, cream[c]	61	(Alsterlund *et al.* 1995)
1995	USA	Vermont, NH		O:8	Pasteurized milk	10	(Ackers *et al.* 2000)
1996	India	Tamil Nadu		4/O:3	Buttermilk	25	(Abraham *et al.* 1997)
2001	USA	Tennessee		4/O:3	Chitterlings	12	(Jones 2003)
2002	Croatia-Italy		Oil tanker	4/O:3	nd	22	(Babic-Erceg *et al.* 2003)
2004	Finland	Kotka		4/O:3	Lettuce[c]	30	
2004	Japan	Nara Prefecture	Nursery school	1B/O:8	Salad	42	(Sakai *et al.* 2005)
2005	Norway			2/O:9	Brawn/ pork chops	11	(Grahek-Ogden *et al.* 2007)
2005	Austria			O:3	Raw milk/?[c]	6	(Much *et al.* 2007)
2006	Norway			4/O:3	Brawn	4	(Tafjord Heier *et al.* 2007)
2006	Japan			2/O:9	nd	2	(Moriki *et al.* 2010)
2009	Finland	Joensuu	Household	2/O:9	nd	4	unpublished data, THL
2009	Australia	ACT	Restaurant	nd	Barbeque Pork[c]	3	(Anonymous 2009)
2010	Sweden	Göteborg	Barbecue party	nd	Poorly cooked pork	130	(Anonymous 2011)
2010	Austria			2/O:9	nd	2	(Sagel and Pekard-Amenitsch 2011)
2010	Finland	Leppävirta	School,nursery	2/O:9	Grated carrots [c]	42	(Huovinen and Sihvonen 2011)
2011	Norway			2/O:9	Bagged salad mix	21	(Macdonald *et al.* 2011)

a) nd = not determined; b) two separate outbreaks; c) vehicle not isolated

2.5 Isolation and cultivation of *Y. enterocolitica* from clinical specimen

Isolation of an infectious strain is a cornerstone of bacterial identification in clinical microbiology. The most common solid selective media used in clinical microbiology laboratories for the isolation of *Y. enterocolitica* is cefsulodin-irgasan-novobiocin agar (CIN) (Schiemann 1979). Due to D-mannitol fermentation *Yersinia* strains form bulls-eye like colonies on agar (Schiemann 1979). CIN agar has been shown to be superior to the alternatives of MacConkey (MAC), Salmonella-Shigella (SS), cellobiose-arginine-lysine (CAL) agars in recovery of *Y. enterocolitica* from fecal samples (Head *et al.* 1982). Also, other *Yersinia* ssp., as well as *Citrobabter* ssp., *Serratia* ssp., *Proteus* ssp., *Aeromonas*, *Morganella* ssp., and *Enterobacter agglomerans*, can form bulls-eye colonies on CIN (Schiemann 1979; Devenish and Schiemann 1981; Harmon *et al.* 1983; Janda and Abbott 2010). Chromogenic agars such as YeCM (Weagant 2008), and YECA (Denis *et al.* 2011) were able to separate pathogenic BT 1B, as well as BTs 2-4, from *Y. enterocolitica* BTs from BT 1A and background flora. Recently, CHROMagar Yersinia (CAY) for the presumptive detection of virulent *Y. enterocolitica* from human stools was introduced (Renaud *et al.* 2013).

2.5.1 Cold-enrichment

Isolation of *Yersinia* from faecal or other samples is not always successful because of the overgrowth of competing *Enterobacteriaceae*. To increase the yield of *Yersinia*, samples are cold-enriched at 4° C in phosphate-buffered saline (PBS) has been used (Greenwood *et al.* 1975; Toma and Deidrick 1975). Also different broths and agars have been used for cold-enrichment (Weissfeld and Sonnenwirth 1980). On the other hand, in several studies, it has been advised to avoid cold-enrichment since it increases number of BT 1A and other *Yersinia* ssp. isolates (Pai *et al.* 1979; Marks *et al.* 1980; Van Noyen *et al.* 1980; Weissfeld and Sonnenwirth 1980; Van Noyen *et al.* 1981; Ratnam *et al.* 1982). However, significantly increased numbers of *Y. enterocolitica* from humans are gained by cold-enrichment (Kontiainen *et al.* 1994). In other study three weeks cold-enrichment in PBS increased isolation rate considerably in asymptomatic subjects, but only minimally in patients with diarrhoea (Pai *et al.* 1979). Cold-enrichment has also been shown to be significantly more efficient than direct plating or selective enrichment for isolating *Y. enterocolitica* also from pigs (Van Damme *et al.* 2013).

2.6 Phenotypic identification of *Y. enterocolitica*

The phenotypic identification of *Y. enterocolitica* is traditionally done based on the ability of a strain to metabolise selected substrates as shown in Table 1 (Wauters *et al.* 1987; Wauters *et al.* 1988; Robins-Browne 2007). Commercial identification

systems, such as Api20E, Api Rapid 32 IDE, Micronaut E, are also available. Comparison of available identification systems found Api20 E the most sensitive and cost-efficient method for *Y. enterocolitica* identification at the species level (Neubauer *et al.* 1998). More reliable results can be obtained if Api20E is incubated at 28 ℃ instead of 37 ℃ (Archer *et al.* 1987). Also the test cards of Vitek GNI and Vitek2 are used for identification of *Yersinia* (Linde *et al.* 1999; Crowley *et al.* 2012). Recently a method for differenting the BT 1A from pathogenic BTs by detection β–glucosidase activity was introduced (Karhukorpi and Päivänurmi 2013). Fourier Transform Infrared (FTIR) Spectroscopy is a technique that provodes information about the biochemical composition of the bacterial strain. It has been successfully applied to identification of *Y. enterocolitica* BTs 1A, 2/O:9, 2/O:5 and 4/O:3, as well as *Y. bercovieri, Y. intermedia* and *Y. rohdei* (Kuhm *et al.* 2009). Recently, Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF) based on identification of a specific protein profile of each bacterial strain, has become more and more popular in microbiology. It has been incorporated as an accurate and rapid method for identifying *Y. enterocolitica* strains (Ayyadurai *et al.* 2010; Lasch *et al.* 2010; Stephan *et al.* 2011). However, also problems with separating BT 1B from BT 1A by MALDI-TOF has been reported (Rizzardi *et al.* 2013).

Serological O-antigen typing is an established method used in laboratories for the identification of bacteria. With *Y. enterocolitica* it helps to identify the pathogenic STs and is epidemiologically useful. The most common pathogenic isolates represent STs O:3, O:9, O:5,27 and O:8. The synthesis of O-antigen in *Y. enterocolitica* is regulated by temperature and therefore colonies appear smooth and express complete O-antigen side chains when grown at temperatures below 30 ℃ and rough at 37 ℃ (Skurnik and Toivanen 1993; Bottone 1997). It is also known that *Y. enterocolitica* O-antigen O:9 crossreacts with *Brucella* sp. antigenic determinants interfering in *Brucella* diagnosis (Weynants *et al.* 1996). At least 44 flagellar H-antigens have been described for *Y. enterocolitica* and related species but serotyping of these are not used in routine diagnostics (Aleksic *et al.* 1986; Aleksic and Bockemuhl 1987; Aleksic 1995).

A simple phenotypic test for virulence of *Y. enterocolitica* stains harbouring pYV plasmid is Congo-red magnesium oxalate (CR-MOX) agar (Riley and Toma 1989). It is based on Congo-red uptake and the calcium dependency of strains that carry pYV (Prpic *et al.* 1983). Congo red has a three-dimensional configuration resembling hemin (Kay *et al.* 1985) and the pYV-positive strains produce small red colonies always accompanied by larger white colonies on a CR-MOX (Figure 2). It has been shown that the freshly isolated cultures do not lose the pYV plasmid easily in contrast to strains that are stored or subcultured several times (Farmer *et al.* 1992).

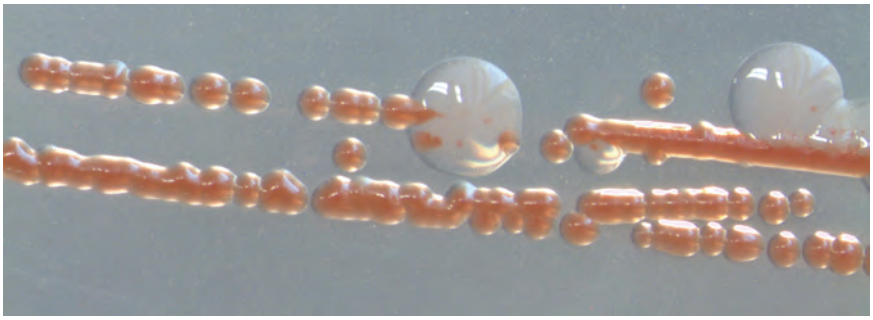

Figure 2. *Y. enterocolitica* 4/O:3 strain growing on CR-MOX (24h, 37°C). Small red colonies represent congo-red uptake, bigger white colonies have lost the virulence plasmid pYV. BT 1A strains form large white colonies on CR-MOX.

2.7 Molecular identification of *Y. enterocolitica* strains

Yersinia phylogeny based on 16S rRNA gene was introduced in the 1990s (Ibrahim *et al.* 1993; Ibrahim *et al.* 1997). A PCR method for differentiating between two subspecies of *Y. enterocolitica* within the genus soon followed (Neubauer *et al.* 2000). The first published whole genome sequence of *Y. enterocolitica* was that of the highly virulent ssp. *enterocolitica* BT 1B (Thomson *et al.* 2006). Today, several whole genome sequences of different *Y. enterocolitica* and *Y. enterocolitica* –like species are available allowing the thorough study of evolution and genetics (Chen *et al.* 2010; Fuchs *et al.* 2011; Wang *et al.* 2011; Klinzing *et al.* 2012; Garzetti *et al.* 2013; Savin *et al.* 2013). A sophisticated whole genome comparison of *Y. enterocolitica* included 100 isolates all representing isolates from all BTs (Reuter et al. 2012). The study showed that species *Y. enterocolitica* are divided into three larger groups, BT 1A, BT 1B and BTs 2-5, and within these larger groups, strains were closely related based on ST (Reuter et al. 2012).

A real time PCR approach based on the chromosomal *ail* gene has been widely used for detecting *Y. enterocolitica* in food and porcine samples (Fredriksson-Ahomaa *et al.* 2007; Fukushima *et al.* 2007; Lambertz *et al.* 2008; Lambertz *et al.* 2008; Wesley *et al.* 2008; Messelhausser *et al.* 2011) Buoyant density gradient centrifugation has been used to remove compounds that inhibit PCR and to prevent false-positive results due to DNA originating from dead cells (Fukushima *et al.* 2007). Further, clinical samples have been investigated with real time PCR (Zheng *et al.* 2007; Wesley *et al.* 2008; Zheng *et al.* 2008).

Many different multiplex PCR applications for detecting *Y. enterocolitica* genes have been introduced (Ibrahim *et al.* 1992; Weynants *et al.* 1996; Thisted Lambertz and Danielsson-Tham 2005). Recently, a quantitative PCR detecting method for nine pathogens including *Y. enterocolitica* was introduced (Antikainen *et al.* 2013). Furthermore, the xTAG Gastrointestinal Pathogen Panel by Luminex

is able to identify *Y. enterocolitica* among 15 pathogens (Kahlau *et al.* 2013; Navidad *et al.* 2013).

2.8 Molecular subtyping of *Y. enterocolitica* strains

In epidemiological studies molecular subtyping of bacteria is essential in surveillance and outbreak investigations. Reproducibility and high discriminatory power are the main features of efficient typing methods. Pulsed-field electrophoresis (PFGE) was developed in the eighties (Schwartz and Cantor 1984) and was soon adopted to epidemiological studies (Arbeit *et al.* 1990). Of the molecular based typing methods based on restriction of DNA, PFGE has so far been the most used method for subtyping for *Y. enterocolitica* strains (Buchrieser *et al.* 1994; Najdenski *et al.* 1994; Saken *et al.* 1994; Iteman *et al.* 1996; Asplund *et al.* 1998; Asplund *et al.* 1998; Fredriksson-Ahomaa *et al.* 1999; Fredriksson-Ahomaa *et al.* 2001; Marranzano *et al.* 2003; Korte *et al.* 2004; Iwata *et al.* 2005; Thisted Lambertz and Danielsson-Tham 2005; Falcao *et al.* 2006; Baumgartner *et al.* 2007; Wang *et al.* 2009). However, the pitfalls of PFGE, when applied to *Y. enterocolitica*, are the large number of bands, the global homogeneity of the pulsotypes and the weak comparability of PFGE patterns between the laboratories (Najdenski *et al.* 1994; Saken *et al.* 1994; Iteman *et al.* 1996; Asplund *et al.* 1998; Fredriksson-Ahomaa and Korkeala 2003; Fredriksson-Ahomaa *et al.* 2006). The use of more than one restriction enzyme has been shown to give a better resolution in PFGE (Fredriksson-Ahomaa *et al.* 2006). Later, the Multiple Locus Variable number of tandem repeat Analysis (MLVA) has shown to have excellent reproducibility for many bacterial pathogens and therefore it is well-suited for interlaboratory comparisons (Lindstedt 2005). MLVA has shown to offer notably higher discriminatory power for *Y. enterocolitica* subtyping (Gierczyński *et al.* 2007; Gulati *et al.* 2009; Wang *et al.* 2012; Virtanen *et al.* 2013). Different molecular methods used for subtyping *Y. enterocolitica* are listed in the Table 5.

Table 5. Examples of different molecular methods used for subtyping *Y. enterocolitica.*

Method	Based	Referenc
MLEE	Electrophoretic mobilities of cellular enzymes	(Dolina and Peduzzi 1993)
PFGE	Electrophoresis of genomic DNA cut by rare-cutting restriction enzymes in an alternating voltage gradient	(Najdenski *et al.* 1994)
RAPD	PCR of genomic DNA with random, short primers	(Rasmussen *et al.* 1994)
PCR-ribotyping	PCF amplification followed by restriction of 16S and 23S rRNA	(Lobato *et al.* 1998)
YeO:3RS probe	Hybridising of YeO:3RS probe in region in pAY100	(Hallanvuo *et al.* 2002)
Polymorphic Tandem Repeats	PCR with primers targeting Variable Number Tandem Repeat (VNTR) of CCAGC	(de Benito *et al.* 2004)
REP/ERIC PCR	PCR with primers targeting of repetetive extragenic palindromic (REP) and enterobacterial repetitive intergenic consensus (ERIC) elements	(Sachdeva and Virdi 2004)
AFPL	PCR amplification of restriction fragments from a total digest of genomic DNA	(Kuehni-Boghenbor *et al.* 2006)
MLVA	Multiple loci VNTR differences are detected	(Gierczyński *et al.* 2007)
RFLP (gyrB)	PCR amplification followed by restriction digestion	(Gulati and Virdi 2007)
RFLP (blaA, blaB)	PCR amplification followed by restriction digestion	(Bonke *et al.* 2011)

2.9 Antimicrobial resistance

The increase of multidrug resistance in *Enterobacteriaceae* has become major healthcare concern worldwide. The molecular mechanisms by which bacteria develop resistance to antimicrobials are extensive. The most frequent type of the acquiring antimicrobial resistance is by horizontal gene transfer via the conjugation of a plasmid (Alanis 2005). Some bacteria are also naturally resistant to some antibiotics. *Y. enterocolitica,* for instance, produces naturally ß-lactamase enzymes which provide resistance to ß-lactam antibiotics such as ampicillin, carbenicillin, penicillin, and first-generation cephalosporins (Pham *et al.* 1991; Stock *et al.* 1999; Pham *et al.* 2000). *Y. enterocolitica* strains have mainly been reported to have high susceptibility to antimicrobials (Prats *et al.* 2000; Mayrhofer *et al.* 2004; Baumgartner *et al.* 2007; Bucher *et al.* 2008; Bhaduri *et al.* 2009). However, also multiresistance of *Y. enterocolitica* strains has been described (Capilla *et al.* 2003; Sanchez-Cespedes *et al.* 2003; Falcao *et al.* 2006). The antimicrobial resistance of *Y. enterocolitica* has not been monitored regularly in Finland although the surveillance of antimicrobial resistance would be useful for epidemiological studies. In the 90's *Y. enterocolitica* strains in Finland were found to be mainly susceptible to most antimicrobials (Kontiainen *et al.* 1994).

3 Aims of the Study

The aims of this study were:

1) to investigate the occurrence of different *Y. enterocolitica* BTs/STs and *Y. enterocolitica* -like species in clinical human specimens in Finland

2) to evaluate the methods used for the identification of *Y. enterocolitica* strains isolated from clinical human specimens in clinical microbiology laboratories in order to improve accurate reporting to NIDR

3) to develop methods for subtyping of *Y. enterocolitica* strains suitable for epidemiological outbreak investigations

4) to analyse the role of different *Y. enterocolitica* BTs/STs in association with clinical picture of the patients and to identify sources of infection

5) to genotype *Y. enterocolitica* BT 1A strains and study differences in their potential pathogenicity

4 Materials and Methods

The techniques used in this thesis are listed in the Table 5 and materials in the Table 6. Materials and methods are described in detail in each article I-V. The bacterial strains are listed in the Appendix 1.

4.1 Study Design

All the 23 Finnish clinical microbiology laboratories were asked about the methods they used isolating and cultivating *Y. enterocolitica*. Based on their answers seven laboratories were selected. Each adhered to similar methology, namely 5-7 days cold-enrichment in peptone broth included in their *Yersinia* isolation procedure were selected to study (Figure 3). Three additional laboratories that did not use cold-enrichment were added in the study to increase the number of strains. Together these 10 laboratories isolate approximately 2/3 of all *Y. enterocolitica* strains reported in Finland annually. The laboratories sent all *Yersinia*, except *Y. pseudotuberculosis,* strains isolated between the 1st January and 31th December 2006 by post to the Enteric Bacteria Laboratory (EBL) of the National Public Health Institute (presently the Bacteriology Unit of the National Institute for Health and Welfare) for the study. EBL provided strain identification to the laboratories in real time if it was requested.

Along with a strain, laboratories also sent the primary plate from which isolation had been made and other information about the sample; methods used, a semi-quantitative estimate of bacterial amount in sample, if another pathogen was found in the same sample and other relevant information, e.g. trips abroad - if the data was available. Stool samples were simultaneously tested for *Salmonella*, *Shigella* and *Campylobacte*r in the sending laboratories. The laboratories also detailed the number of all stool samples studied and other enteropathogens found during 2006. All together 472 *Yersinia* strains and 308 primary plates strains were collected and studied with different phenotypic and genotypic methods (Table 5**).** Antimicrobial susceptibitity testing with was performed by agar diffusion technique using an epidemiological set of 12 antimicrobials. A strain resistant to at least four antimicrobials was called multiresistant.

A detailed questionnaire was sent to each patient to ascertain age-, gender, and geographically matched controls immediately after their *Yersinia* isolate was received by EBL to collect data for the case-control study of symptoms and sources (Article II). Cases with some other bacterial pathogen or previous *Yersinia* isolate within half a year prior the study or no matching controls were excluded from the

case-control study. Thus 295 cases and 1002 controls were included in the case-control study. In addition, 200 stool samples from healthy people were studied by cold enrichment for possible *Yersinia* strains.

Table 6. The methods used in the study. The Roman numerals refer to the original articles in which the methods were applied and described in detail.

Method	Article
Cold-enrichment of *Yersiniae*	I
Bio- and serotyping	I
Colony morphology on CIN-agar	I
CR-MOX –agar test for virulence	I
Partial16S rRNA gene sequencing	I, V
gyrB gene sequencing	I, V
Case-control questionnaire survey	II
Two-sample t-tests	II
Chi square test	II, III
Antimicrobial susceptibitity testing with agar diffusion technique and minimal inhibitory concentration (MIC) tests	III
Conjugation experiment of a resistance plasmid	III
lcrE gene detection by PCR	III
Inv gene detection by PCR	III
gyrA-gene sequencing	III
Multilocus variable tandem repeat analysis (MLVA) with VNTR loci of V2A, V4, V5, V6, V7 and V9	III
Pulsed-field gel electrohoresis (PFGE) with *Not*I	III
ystA gene detection by PCR	III
ystB gene detection by PCR and sequencing	III
ail gene detection by PCR and sequencing	III, IV
Bayesian Analysis of Population Structure (BAPS)	V
LPS-typing	V
Multilocus sequence typing (MLST) of genes *adk, argA, aroA, glnA, gyrB, thrA, trpE*	V
Phage sensitivity assay with bacteriophages	V
Serum complement killing assay	V

Table 7. The materials used in the study. The Roman numerals refer to the original articles in which the methods were applied and described in detail.

Materials	Number	Article
Clinical strains	462	I, III, IV, V
Information of the strains[a]	462	1
Primary plates[b]	308	I
Stool samples from healthy people	200	I
Cases in case-control study	295	II
Controls in case-control study	758	II
Environmental strains	1	IV

[a] Information about the sample, methods and temperatures used for isolation, bacterial amount in a sample by detecting growth on four streaking areas, other bacterial anteropathogens isolated year 2006

[b] Primary culturing CIN plates from where the *Yersinia* strain was isolated with four typical colonies circle

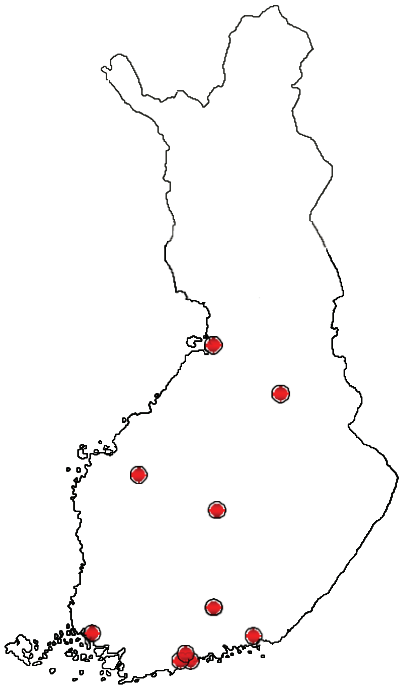

Figure 3. Geographical location of the 10 clinical microbiology laboratories from which 473 *Yersinia* strains were collected for the study in 2006.

5 Results

5.1 Isolation and identification of *Y. enterocolitica* (I, unpublished)

All the 10 clinical microbiology laboratories used direct plating on CIN. In addition, seven laboratories also used 5-7 days' cold-enrichment in peptone broth at 4-6 °C prior to plating on CIN. Cold-enrichment increased the number of all *Yersinia* isolates. Of the pathogenic BTs 4/O:3 and 2/O:9, 28%; of BT 1A 73%; *Y. enterocolitica* -like species 85%, were only found by cold-enrichment (Figure 4). All the strains isolated by direct plating were also always isolated by cold-enrichment. The cases of 4/O:3 find only by cold enrichment (n=15). Of these eight out of 10 patients had reported diarrhoea as one of the symptoms (from five patients the symptoms were not known). For instance, a strain of *Y. enterocolitica* 4/O:3 isolated from two stool samples of a 10-months old child taken 19 days apart from another, could both times only be found by cold-enrichment.

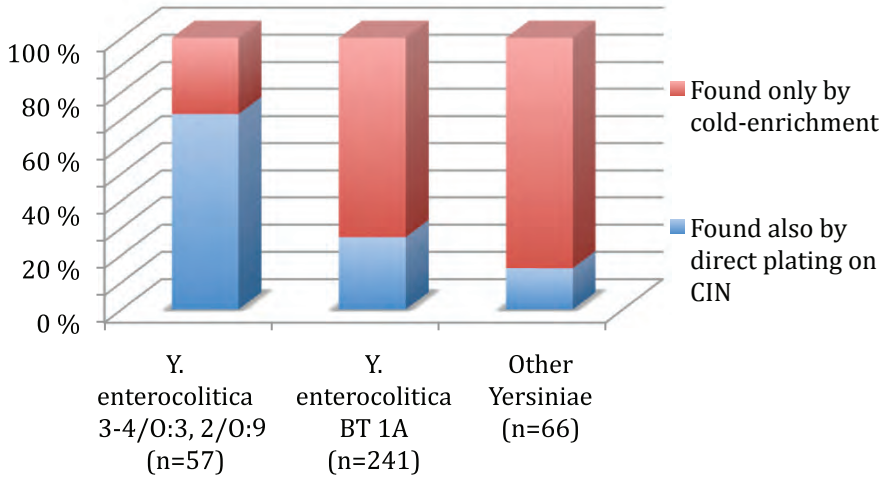

Figure 4. Strain isolation rates when parallel direct plating on CIN and 5-7 days' cold-enrichment in peptone broth at 4-6 °C prior to plating on CIN. This was applied for 364 fecal samples.

When, 308 primary CIN plating's of the stool samples were studied for possible co-existence of different *Yersinia* strains, 10 events were found (Table 8). Of these cases 5 were only recovered by cold-enrichment.

Table 8. Other *Yersinia* strains found when two additional colonies were studied from the primary CIN plates of 308 faecal samples.

Primary isolate in Routine laboratory	Secondary isolate in Reference laboratory	Number	Isolates found only by cold-enrichment
***Y. enterocolitica* BT 1A**	*Y. kristensenii*	2	0
***Y. enterocolitica* BT 1A**	*Y. frederiksenii*	1	0
***Y. enterocolitica* BT 1A**	*Y. intermedia*	1	0
***Y. enterocolitica* BT 1A**	*Y. mollaretii*	1	1
***Y. enterocolitica* 4/O:3**	*Y. kristensenii*	1	0
Y. bercovieri	*Y. enterocolitica* BT 1A	3	3
Y. frederiksenii	*Y. enterocolitica* BT 1A	1	1

There were also 20 cases when another pathogenic bacterium was isolated along with a *Yersinia* strain in the laboratories (Table 9). Prevalence of other pathogens in fecal samples with *Y. enterocolitica* pYV+ strains, BT 1A, and other *Yersinia* ssp. were 5.1%, 4.7%, and 5.2%, respectively.

Table 9. Bacterial pathoges found in the fecal samples with *Yersinia* isolate.

Yersinia isolate	With other pathogen	Number*	Cold-enrichment*
***Y. enterocolitica* BT 1A**	*Campylobacter jejuni*	6	3
***Y. enterocolitica* BT 1A**	*Salmonella* Enteritidis	1	1
***Y. enterocolitica* BT 1A**	*Salmonella* Corvallis	1	0
***Y. enterocolitica* BT 1A**	*Aeromonas* sp.	1	1
***Y. enterocolitica* BT 1A**	*Clostridium* sp.	1	1
***Y. enterocolitica* BT 1A**	*Shigella flexnerii*	1	0
***Y. enterocolitica* BT 1A**	*Staphylococcus aureus*	1	1
***Y. enterocolitica* 4/O:3**	*Campylobacter jejuni*	2	0
***Y. enterocolitica* 4/O:3**	*Salmonella* Braenderup	1	1
***Y. enterocolitica* 4/O:3**	*Salmonella* Haifa	1	1
Y. bercovieri	*Campylobacter jejuni*	2	1
Y. frederiksenii	Salmonella Typhimurium	1	1
Y. mollaretii	*Clostridium perfringens*	1	1

*Number of *Yersinia* isolates found only by cold-enrichment from a samples with another bacterial pathogen.

Patients with double infections were excluded from the case-control study, since the causative agent behind symptoms was unclear. Travelling abroad was reported by 16 out of 18 patients with double infection.

The initial identification of the *Yersinia* findings carried out in the laboratories were compared with the results after all feno- and genotypic tests in the reference laboratory. Pathogenic BTs were correctly identified to be *Y. enterocolitica* in 94% of the strains. In 2% (n=7) of the BT 1A strains were suspected to belong to pathogenic serotypes O:3 or O:9. Correct identification of serotypes O:5 and O:8 were done with 44/98 strains, however, all these strains were later assigned to BT 1A.

5.2 Occurrence and characteristics of clinical isolates of *Y. enterocolitica* and related species in Finland (I, III)

In 2006, 473 *Yersinia* strains from 462 patients were isolated from all together 41 848 clinical stool cultures were studied from 10 Finnish clinical microbiology laboratories enrolled in the study. Pathogenic bio/serotypes 3-4/O:3 and 2/O:9 constituted 18% of these strains (Figure 5). All these pathogenic strains of *Y. enterocolitica* were positive in the phenotypic virulence test with CR-MOX agar and had the genomic and plasmid-associated virulence markers of *ail* and *lcrE* in the PCR tests.

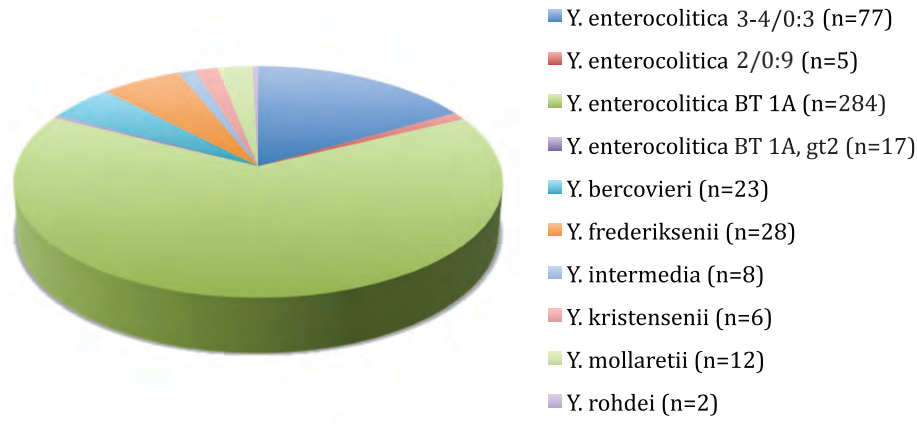

Figure 5. 462 strains isolated in 2006 from 462 Finnish patients of the study. 17 strains originally identified as *Y. enterocolitica* BT 1A but later were found to differ distinctively from *Y. enterocolitica* in MLSA and 16S rRNA analysis.

The strains of pathogenic bio/serotypes showed overall homogeneity by PFGE with *NotI*. More resolution was gained by 6-loci MLVA: 82 strains of BTs 3-4/O:3 and 2/O:9 isolated in 2006 were divided into 77 different MLVA-types (Article III). The MLVA typing was also applied to strains associated with an outbreak in the year

2003 and confirmed that strains of a foodborne outbreak were indeed caused by multiresistant 4/O:3 strain.

The majority of strains, 64%, were identified as *Y. enterocolitica* BT 1A strains by biotyping at the reference laboratory. Two strains were non biotypeable (NBT), but where assigned to *Y. enterocolitica* based on their 16S rRNA *Y. enterocolitica* -like strains constituted 18% of clinical *Yersinia* isolates. These strains were assigned to the species *Y. bercovieri, Y. frederiksenii, Y. intermedia, Y. kristensenii, Y. mollaretii* and *Y. rohdei* (Figure 5) by biotyping and 16S rRNA sequencing.

5.3 Characteristics of *Y. enterocolitica* BT 1A strains (I, IV, V)

The subset of 71 strains of *Y. enterocolitica* BT 1 A and NBT strains in the multi-locus sequence typing (MLST), 16S rRNA sequencing, LPS-analysis, PCR for *ystA* and *ystB*, phage typing, human serum complement killing assay and analysis of the symptoms of the patients differed in their pathogenicity-related properties. Genetic analysis revealed that 17 (6%) of *Y. enterocolitica* BT 1A strains, were identified and formed a subtype cluster with 98% 16S rRNA similarity, separate from the of majority BT 1A strains (Article V). These strains are here called the BT 1A "Genetic group 2", while the majority of BT 1A are called "Genetic group 1". The two *Y. enterocolitica* NBT strains that showed no salicin fermentation or esculin hydrolysis, did not vary genetically from the majority of BT 1A strains.

None of the BT 1A strains had plasmid-associated virulence marker *lcrE*, but one of the strains had the gene *ail* which has a sequence similarity of 99.7% with the *ail*-gene of highly virulent BT 1B strains (Article IV). According to MLST and 16S rRNA analysis this *ail*- positive BT 1A strain was genetically similar to the majority of BT 1A strains and did not differ biotypically or did not show activity in the serum killing assay. The most commonly detected LPS types were similar to the LPS types of reference strains with serotypes O:6,30 and O:6,31 (37%), O:7,8 (19%) and O:5 (15%).

The strains of the Genetic group 2 were uniform in their pathogenicity-related properties: they lacked the *ystB* gene, belonged to the same LPS subtype or were of rough type, were all resistant to five tested yersiniophages, were largely resistant to the serum complement and did not ferment fucose. The strains (n=54) assigned into Genetic group 1 showed much more variation in these properties.

5.4 Antimicrobial susceptibility of clinical *Yersiniae* strains (III, unpublished data)

Of the pathogenic *Y. enterocolitica* bio/serotypes 3-4/O:3 and 2/O:9 (n=80), all were resistant to ampicillin and 19% of the 80 were resistant to four or five of the antimicrobials when a set of 12 antimicrobials tested in an agar disk diffusion test. Three of the strains were resistant to nalidixic acid and showed also lowered sensitivity (0.25, 0.25, or 0.5 mg/L) to ciprofloxacin in minimum inhibitory concentration (MIC) test. All the strains showing resistance to nalidixic acid had required resistance via point mutation in gyraseA (*gyrA*) gene. In a conjugation a strain of bio/serotype 4/O:3 strain resistant to AMP, CHL, STR, SUL, and NAL, was able to transfer the CHL, STR, and SUL resistances in a 30-49 kb -plasmid to strain YeO3-U by conjugation. Travel abroad was significantly (p=0.002) associated with the antimicrobial multiresistance: 34% of the patients with and 5% of the patients without a trip abroad had a multiresistant *Y. enterocolitica* strain.

Y. enterocolitica BT 1A strains were in general susceptible to the tested antimicrobials, except ampicillin and mecillinam (Table 10). *Y. enterocolitica* –like strains were mostly resistant to ampicillin, but single strains showed resistance also to chloramphenicol, streptomycin, sulfonamide, tetracycline, trimethoprim, cefotaxime and/or mecillinam. All BT 1A and *Y. enterocolitica* strains were susceptible to ciprofloxasin, gentamicin, nalidixic acid and imipenem.

Table 10. Antimicrobial susceptibilities (%) of tested *Y. enterocolitica* and *Y. enterocolitica* -like strains isolated in 2006.

	AMP[1]	CHL	STR	GEN	SUL	TET	TMP	CIP	NAL	CTX	MEC	IMI
Y. enterocolitica 4/O:3 (n=75); R[2], (I)	100	17	19, (48)	-	20	7	-, (11)	0, (3)	4	-	1	-
Y. enterocolitica 3/O:3 (n=2); R, (I)	100	100	100	-	100	50			-	-	100	-
Y. enterocolitica 2/O:9 (n=5); R, (I)	100	-	-	-	-	-	-	-	-	-	-	-
Y. enterocolitica BT 1A (n=117); R, (I)	100	-	-	-	1, (9)	-	-	-	-	-, (0.9)	21, (52)	-
Y. bercovieri (n=23); R, (I)	96	-	4	-	16, (35)	-	-	-	-	-, (35)	52	-
Y. frederiksenii (n=27); R	100	4	-	-	-	-	-	-	-	-	41	-
Y. intermedia (n=8); R	86	-	-	-	-	-	-	-	-	-	13	-
Y. kristensenii (n=6); R	67, (17)	-	-	-	17	-	-	-	-	-	-	-
Y. mollaretii (n=12); R ,(I)	83, (17)	-, (25)	-	-	17		-, (25)	-	-	-, (25)	6	-
Y. rohdei (n=2); R	100	100	-	-	-	-	-	-	-	-	50	-

[1]AMP = ampicillin; CHL = chloramphenicol; STR = streptomycin; GEN = gentamicin; SUL = sulfonamide; TET = tetracycline; TMP = trimethoprim; CIP = ciprofloxasin, CTX = cefotaxime; MEC = mecillinam; IMI = imipenem.

[2]R = resistant, I = intermediate

5.5 Symptoms and sources of *Y. enterocolitica* infections (II)

Patients infected with *Y. enterocolitica* 3-4/O:3 and 2/O:9 were younger and had fever more often than those with the BT 1A isolate. On the other hand, patients with BT 1A, suffered more from vomiting. Children under 3-years represented 20% of the patients with pathogenic biotypes, whereas only 0.7% of the patients with BT 1A where under 3-years old. With six patients, BT 1A infection was also shown to be persistent, since strains were isolated from the same patients at intervals of over 20 days. Symptoms of ReA were reported by 10% of the patients with *Y. enterocolitica* 3-4/O:3 and 2/O:9 , 3% of *Y. enterocolitica* BT 1A and 0.3% of the controls. Eating or tasting raw or medium done pork was a significant risk factor for 4/O:3 and 2/O:9 infection (OR 6.6; 95% CI 1.7-24.9), as well as eating in a canteen (OR 3.5; 95% CI 1.6-7.9). Imported fruits and berries were associated with an increased risk of *Y. enterocolitica* BT 1A. When 200 stool samples from healthy people were studied, one *Y. enterocolitica* BT 1A strain was isolated.

5.6 Seasonal variation on *Yersinia* and other enteropathogenic bacteria in clinical stool samples (unpublished data)

In the 2006, the total number of all Yersinia isolations was the highest in May. However, the infections with pathogenic *Y. enterocolitica* bio/serotypes 3-4/O:3 and 2/O:9 were at the highest during September (n=10) and October (n=10). The highest monthly number of *Salmonella* findings was detected in August. In the year 2006, 18% of the *Salmonella* cases were domestic isolates. Infections caused by *Campylobacter* were the most common in July-August. The seasonal peak in the number of cases for BT 1A strains appeared in May (n=48). The Figure 6 illustrates the occurrence of *Yersinia*, *Salmonella* and *Campylobacter* strains found when 41 848 clinical stool cultures in 10 Finnish clinical microbiology laboratories were studied in the year 2006. In the Figure 7 it is shown the monthly occurrence of BT 1A isolates and laboratory confirmed Norovirus cases notified in the NIDR.

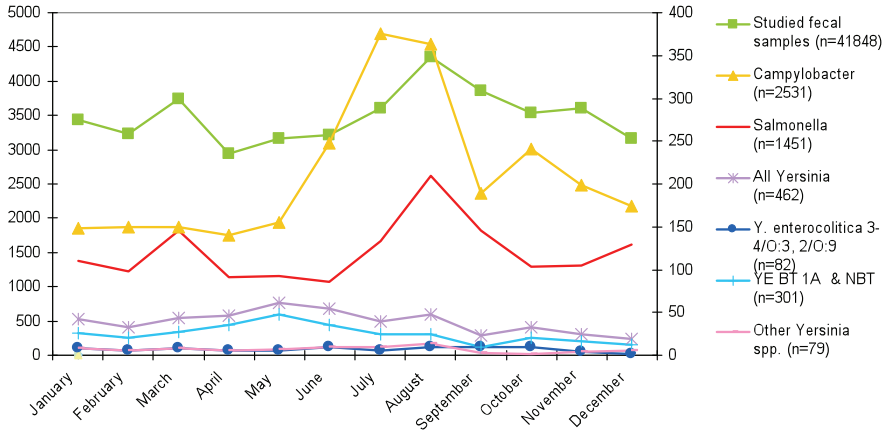

Figure 6. Occurrence on *Yersinia, Salmonella, Campylobacter* in relation to studied faecal samples in the year 2006 in 10 clinical microbiology laboratories enrolled in the study.

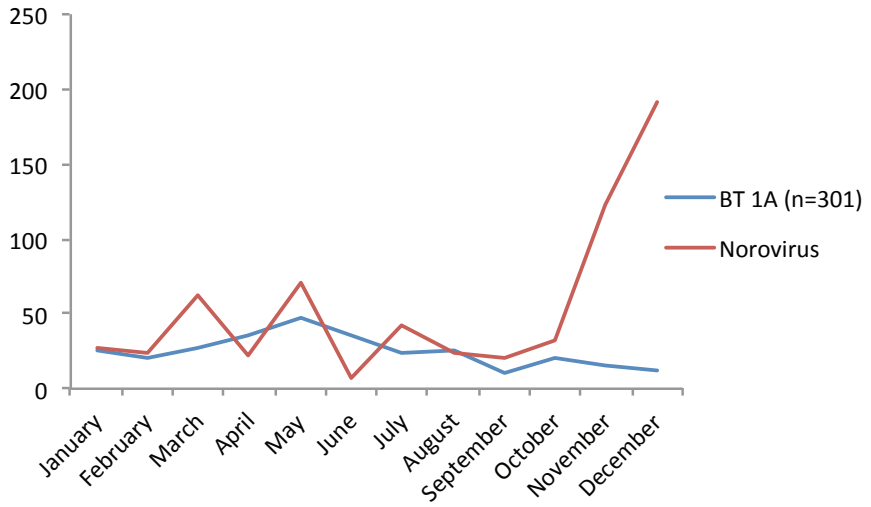

Figure 7. Occurrence on *Y. enterocolitica* BT 1A and laboratory confirmed Norovirus cases notified in the NIDR in 2006.

6 Discussion

6.1 Prevalence and notification of *Y. enterocolitica* in Finland

The proportion of classical pathogenic bio/serotypes 3-4/O:3 and 2/O:9 collected in collaboration with 10 clinical microbiology laboratories in 2006 was only 18% of all the isolates. Interestingly, BT 1A was the predominant (65%) of the clinical strains. In a study in Great Britain 1999-2000 comparable results were obtained, it was found that 53% of clinical *Y. enterocolitica* isolates were BT 1A strains (McNally *et al.* 2004). Furthermore, in Switzerland, almost 40% of the clinical *Yersinia* isolates were BT 1A strains (Burnens *et al.* 1996).

In estimating the real frequency of pathogenic *Y. enterocolitica* infections, seroprevalence of *Yersinia* antibodies in population has been used. Since detection of *Yersinia* antibodies is usually based on the antibodies of the virulence-associated markers, all patients with *Y. enterocolitica* antibodies have been infected with 4/O:3 or 2-3/O:9 strains. In the late 1990's high frequency of *Y. enterocolitica* O:3 and O:9 antibodies among healthy blood donors was 31% in Finland, and 46% in Germany, which shows that *Yersinia* infections in these both populations were quite common (Mäki-Ikola *et al.* 1997). However, the prevalence of reported yersiniosis cases in 2008 in Finland was 11.5 and in Germany 5.3 (EFSA 2010). In Hungary, the prevalence of *Yersinia* antibodies in healthy adults in 2000 was 10% and in Austria the prevalence of *Yersinia* antibodies in solders was 30% in 2005 (Sonnevend *et al.* 2005; Tomaso *et al.* 2006). Interestingly, the prevalence of *Y. enterocolitica* in and Hungary and Austria in 2005 were only 0.4 and 1.1 confirmed cases per 100 000, respectively (EFSA 2010). It is safe to assume that a large percent of *Y. enterocolitica* infections go unreported due to the acute illness not requiring medical attention or *Y. enterocolitica* remains to be undetected. However, due the differences in laboratory and reporting practices in different countries, the European statistics on *Y. enterocolitica* prevalence are not quite reliable.

Previously, it was assumed that most *Y. enterocolitica* cases in Finland were of domestic origin since pathogenic *Y. enterocolitica* are frequently isolated from Finnish pigs (Fredriksson-Ahomaa *et al.* 2006) and prevalence of *Y. enterocolitica* in Finland has historically been high (EFSA 2005; EFSA 2010). However, the lowered susceptibility to antimicrobials suggested that a large number of *Yersinia* strains are actually imported. And indeed, travelling was quite common among the patients in the study. Factors associated with seeking medical care and submitting a stool sample are not known, but it may be that the travellers returning home with

gastroenteritis are more likely to consult the doctor than subjects suffering from domestic foodborne illness.

The register (the National Infectious disease register; NIDR) in Finland was based solely on the reporting of *Y. enterocolitica* species without without any further specifications, until the year 2010. From the beginning of 2010 clinical laboratories were recommended to use the simple biotyping scheme for *Y. enterocolitica* and notify the BTs of *Y. enterocolitica* species. Currently, approximately 60% of the Finnish clinical microbiology laboratories report on specific BTs. It has been noticed that there is clear regional differences in the prevalence of *Y. enterocolitica* in Finland (Huovinen and Sihvonen 2011). Whether these differences in prevalence are real or derived of diagnostic and/or methodological differences remains unclear. Unfortunately, there is no official and standardised reporting system across EU countries. In May 2012 the reporting system of *Y. enterocolitica* in Sweden was renewed such that all BT 1A strains are now excluded from reporting (Rizzardi *et al.* 2013).

6.2 Detecting and identification of *Yersinia* strains

One thing clearly affecting on *Yersinia* findings is the use of cold-enrichment. The long time required for cold-enrichment has reduced the clinical value of the method (Weissfeld and Sonnenwirth 1980). Nevertheless, without cold-enrichment some of the pathogenic strains remain undetected (Kontiainen *et al.* 1994). A strong argument for using cold-enrichment is that in the present study 28% of the pathogenic 4/O:3 strains were only found by 5-7 days cold-enrichment. This is in contrast with previous studies claiming that cold-enrichment is useless in isolating *Y. enterocolitica* and increases only the isolation number of BT 1A strains and the pathogenic BTs from nonsymptomatic carriers (Pai *et al.* 1979; Van Noyen *et al.* 1980; Van Noyen *et al.* 1981). The pathogenic *Y. enterocolitica* strains in the present study recovered by cold-enrichment, were from patients with diarrhoea. In addition, the loss of pYV was not detected in the studied freshly isolated *Y. enterocolitica* cultures.

In the Finnish laboratories over 50% of the pathogenic BTs were assigned correctly right to ST level and 99% right at species level. Serotyping was used by most of the laboratories in the present study. If a routine laboratory applied biotyping combined with serotyping, strains were mostly correctly identified. However, if only serotyping was combined only with commercial identification system such as Api20E misidentification of pathogenic strains resulted frequently. It was detected that *Y. enterocolitica* BT 1A serotypes O:5 and O:8 are common, whereas bio/serotypes 3/O:5,27 and 1B/O:8, were not isolated in this study and are uncommon in Finnish patients. Therefore many ST O:5 and O:8 were assigned

initially as pathogenic strains. Other methods, such as detecting β–glucosidase activity combined with chromogenic agars or Vitek2 GN-ID card, can be applied to differentiate *Y. enterocolitica* BT 1A from strains of pathogenic BTs (Karhukorpi and Päivänurmi 2013). Even the biotyping of *Y. enterocolitica* has plenty of pitfalls, for instance, in the present study two nontypeable BT 1A strains (negative reaction in esculin hydrolysis and salicin fermentation) were found. Furthermore, it was sometimes impossible to separate certain *Y. frederiksenii* strains by biotyping from BT 1A strains.

In the future, rapid and accurate methods such as MALDI-TOF will be increasingly replacing laborious phenotyping methods in identifying of *Y. enterocolitica* isolates (Ayyadurai *et al.* 2010; Lasch *et al.* 2010; Stephan *et al.* 2011). However, also MALDI-TOF needs evaluation, since *Y. enterocolitica* is such a heterogenic species with different BT/ST combinations that at the moment available protocols do not unambiguously identify all (Rizzardi *et al.* 2013). Considering the metabolic heterogeneity of BT 1A and closely related *Y. enterocolitica* -like strains there probably still be challenges also in MALDI-TOF.

6.3 Detecting disease outbreaks caused of *Y. enterocolitica* 4/O:3 and 2/O:9

For the detecting outbreaks of *Y. enterocolitica*, accurate and inter-laboratory standardised methods are needed. In the present study MLVA method (Gierczyński *et al.* 2007) was further developed by use of fluorescently labeled PCR primers and sizing by an automated DNA sequencer. For the first time, MLVA was applied successfully to subtype *Y. enterocolitica* 4/O:3 strains of a foodborne outbreak and separate the sporadic strains. The MLVA method using 6 loci was found to be superior in accuracy and discriminatory power compared with PFGE using *Not*I. In Norway MLVA has been used to confirm an disease outbreak caused by *Y. enterocolitica* 2/O:9 associated with bagged salad (Lindstedt 2011; MacDonald *et al.* 2012). The recent studies have further confirmed the suitability of this method in epidemiological studies of *Y. enterocolitica* strains (Wang *et al.* 2012; Virtanen *et al.* 2013).

6.4 Risks of *Yersinia* infection and clinical picture

A clear risk-factor for a infection with *Y. enterocolitica* pathogenic BTs 3-4/O:3 and 2/O:9 in the case-control study was eating undercooked or raw pork meat. This is in accordance with previous studies (Satterthwaite *et al.* 1999; Fredriksson-Ahomaa *et al.* 2006). Conversely, risk factors for *Y. enterocolitica* BT 1A infection were imported fruits and berries, as well as eating in a restaurant or cafeteria. The highest

seasonal peak for BT 1A cases was in May. Interestingly, the late spring in 2006 was also the seasonal peak of norovirus. However the highest peak of norovirus case was in December, which did not correlate with BT 1A. Carrots in carrot-processing plants (Määttä *et al.* 2013) and grated carrots and other vegetables in cafeterias have been shown to contain *Y. enterocolitica* BT 1A strains, frequently (Niskanen 2007). The seasonal peak of BT 1A infections in May be able to blend together with possible source of carrots and vegetables. The long storage in cold provides well-suited conditions also for *Y. enterocolitica* BT 1A strains to proliferate, as shown with *Y. pseudotuberculosis* (Rimhanen-Finne *et al.* 2009; Vasala *et al.* 2013).

The patients with BT 1A strains were older than those with pathogenic BTs. There were almost no small children among BT 1A cases, whereas with pathogenic BTs they were overrepresented. On the other hand, association of BT 1A with small children has been reported previously (Morris *et al.* 1991). It can be speculated that the different source of infection and perhaps also higher infective dose with BT 1A than with pathogenic BTs, could affect the reported rate of incidence of BT 1A in children.

The clinical consequence of infections caused by pathogenic BTs and BT 1A differed. Fever is significantly more common with subjects pathogenic BTs, and vomiting is more common in patients with BT 1A. This is in contrast to the study (Burnens *et al.* 1996), where the clinical picture was similar with BT 1A and pathogenic BTs. It has been considered that ystB toxin, analogue to enterotoxin of ETEC, would be most important virulence factor of BT 1A strains. However, the diarrhoea caused by ETEC strains is usually lasts only 1-2 days (Granum 2006). In contrast to that, long-lasting diarrhoea was more common with patients with BT 1A than with pathogenic BTs.

ReA was reported by 10% of the patients of bio/serotypes 4/O:3 and 2/O:9, which consistence with other studies (Rosner *et al.* 2013). Interestingly, also 3% of patients BT 1A compared to 0.3% controls suffered from symptoms of ReA. Reactive arthritis associated with BT 1A strains has been described with a HLA-B27 patient (Ebringer *et al.* 1982). In addition, IgG, IgA and IgM antibodies against a strain of BT 1A/O:6 has been isolated from a patient (Skurnik 1983). The number of ReA caused by BT 1A is hard to estimate, since commonly used commercial *Yersinia* immunoassays detect only antibodies of pathogenic bio/serotypes. Nevertheless, the long-term sequela caused by *Y. enterocolitica* may have more significant costs to individuals and society than the acute yersiniosis.

6.5 Antimicrobial resistance

Although antimicrobials treatment in yersiniosis is rare, the systemic infections and bacteremia associated with *Y. enterocolitica* require antibiotic treatment. Subsequently, further enquiry into antibiotic resistance in *Y. enterocolitica* is needed. In recent years bacterial resistance to commonly used antibiotics has became a major global healthcare problem (Alanis 2005). This study found that multiresistant strains of *Yersinia* with lower susceptibility to second-generation fluoroquinolone, ciprofloxasin, appeared also in Finnish patients. Although yersiniosis is usually self-limiting and does not require treatment, more severe or complicated infections are commonly treated with fluoroquinolones.

6.6 Potential pathogenicity of *Y. enterocolitica* BT 1A

It has been suggested, that *Y enterocolitica* BT 1A strains represent opportunistic pathogens containing virulence-associated factors that might cause an infection with symptoms similar to that caused by the pathogenic BTs, when the host defense is weakened (Batzilla *et al.* 2011). This theory is supported by findings that BT 1A is frequently isolated from asymptomatic subjects. In the present study 0.5% (n=1) contained BT 1A strains when 200 stool samples from healthy people were studied. In fact, the prevalence of BT 1A in diseased people was not much higher, 0.6% (n=271) of samples contained a BT 1A strain when the 41.848 stool samples from diseased were isolated. The patients with BT 1A isolate here reported prolonged duration of the symptoms more often and were unable to define the accurate onset of symptoms. This suggests that these patients with 1A may have some underlying condition, which has made the environment favourable to opportunistic pathogens. For instance, *Y. enterocolitica* findings have been associated with non-steroid anti-inflammatory drug (NSAID) –induced colitis and are proposed to be more consequence rather than cause (Knösel *et al.* 2009). Furthermore, the persistence of BT 1A infection was supported by six patients from whom BT 1A strains were isolated at an interval of three weeks or more.

In a serum resistance assay, some STs showed notable activities: BT 1A/O:5 and BT 1A/ O:6, 87% and 67%, respectively, showed resistance to complement-mediated killing. It has been shown that BT 1A strains were able to survive and proliferate inside macrophages (Grant *et al.* 1999; McNally 2007; Dhar and Virdi 2013). It may be that certain serotypes of BT 1A are more eligible to opportunistic lifestyle than others.

One of the interesting observation of this study is, that also BT 1A strains can harbour chromosomal gene *ail*, which previously has been thought to be present solely in pathogenic strains (Miller *et al.* 1989). However, the BT 1A strain with

gene *ail* did not show any activity in the serum resistance test and it was genetically highly similar to majority of the BT 1A strains. Lately, another study reported a characterisation of a *Y. enterocolitica* BT 1A strain isolated from pork meat in Germany with an identical *ail* gene (Kraushaar *et al.* 2011). It has been suggested that BT 1A is a progenitor of pathogenic BTs (Reuter et al. 2012). Therefore, it is possible that *ail* gene is a reliec in some BT 1A strains. It remains to be seen, whether the *ail* gene has a function in BT 1A strains and is that function related with pathogenicity. In earlier study, when BT 1A strains were transformed with functional *ail* gene from BT 1B strain 8081, *ail* was found to be expressed but it did not enhance the ability of the strains to adhere or invade to tissue culture cell line (Pierson and Falkow 1993).

Y. enterocolitica –like bacterial strains are with low public health significance, like most of the BT 1A strains. The genetic heterogeneity found by MLST analysis and 16S rRNA gene sequencing revealed that *Yersinia frederiksenii* genospecies 2 is *Yersinia massiliensis* (Souza *et al.* 2013Souza *et al.* 2013). Therefore, it is likely that some of the strains of assigned as *Y. frederiksenii* in the present study, are actually *Yersinia massiliensis.*

7 Conclusions and Future Considerations

The use of cold-enrichment, CIN agar and detection of the pYV plasmid have been shown to be successful in isolating pathogenic *Yersinia* BTs strains from human faecal samples. The biotyping plays a key role, but is not sufficient alone for differentiation between BT 1A and *Y. enterocolitica* -like microbes. The use of cold enrichment increased the yield of all *Yersinia* isolates from stool samples and diarrhoeic patients, including but not limited to pathogenic BTs.

The BT/ST notification of *Y. enterocolitica* strains should be applied to all isolates in Finland. If this cannot be applied, it should be considered excluding the BT 1A strains from notification, until it is proved that they do have public health significance. The including BT 1A strains increases the prevalence of *Y. enterocolitica* in Finland compared with other European countries. Of all the patients in the study with *Y. enterocolitica* isolates, over one third had travelled abroad before falling ill with gastroenteritis. This indicates that large number of *Yersinia* infections in Finland is not of domestic origin. At the moment, the statistics on the prevalence of *Y. enterocolitica* at the European level are not comparable between different countries and even less data is available prevalence outside Europe. It would be useful to harmonise notification systems in different EU countries. In addition, monitoring of antimicrobial resistance of *Y. enterocolitica* isolated should be paid attention.

The MLVA method was found to be a powerful epidemiological tool with high discriminatory power and reproducibility in subtyping of sporadic and outbreak related strains of 3-4/O:3. Hence MLVA can be used to replace the PFGE in the surveillance and outbreak investigations of *Y. enterocolitica* BTs 2-5. It would be useful, if the MLVA method would be internationally standardised and results collected in the reference library.

The possession of the chromosomal *ail* -gene by PCR does not guarantee the detection of pathogenic strain, since it was shown that BT 1A can also harbour gene *ail*. Whether gene *ail* has a role in the pathogenesis of BT 1A strains is unclear, but no indication of any enhanced serum complement resistance was found in serum killing assay with a *ail*+ strain in our study. Furthermore, MLST showed that the BT 1A strains in Finland were actually divided into two completely separate genetic groups. The minor genetic group were largely resistant to the serum complement

killing, and were characterized from other BT 1A strains that they lacked the *ystB* gene, were resistant to tested phages and did not ferment fucose.

The symptoms of patients with *Y. enterocolitica* BT 1A differed from those of patient with invasive strains. A significant risk factor for a pathogenic BT *Y. enterocolitica* infection was the consumption of raw or undercooked pork, whereas sources of BT 1A were ambiguous. Attention should be paid to the prevention of the access of *Y. enterocolitica* in the food to prevent the infections in the first place. The possible pathogenicity of *Y. enterocolitica* BT 1A should be further studied taking into account the genetic subgroups among the BT. Especially association with BT 1A strains and ReA should be studied by investigating the antibodies from the patients.

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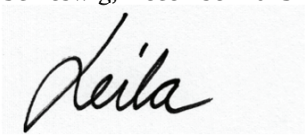

Abraham, M., M. Pai, G. Kang, G. V. Asokan, S. R. Magesh, S. Bhattacharji and B. S. Ramakrishna (1997). "An outbreak of food poisoning in Tamil Nadu associated with *Yersinia enterocolitica*." Indian J. Med. Res. **106**: 465-468.

Ackers, M.-L., S. Schoenfeld, J. Markman, M. G. Smith, M. A. Nicholson, W. DeWitt, D. N. Cameron, P. M. Griffin and L. Slutsker (2000). "An outbreak of *Yersinia enterocolitica* O:8 infections associated with pasteurized milk." J. Infect. Dis. **181**: 1834-1837.

Adjei, A. A., G. K. Kuma, Y. Tettey, P. F. Ayeh-Kumi, J. Opintan, F. Apeagyei, J. O. Ankrah, T. K. Adiku and E. G. Narter-Olaga (2009). "Bacterial contamination of blood and blood components in three major blood transfusion centers, Accra, Ghana." Jpn J Infect Dis **62**(4): 265-269.

Ahvonen, P., K. Sievers and K. Aho (1969). "Arthritis associated with *Yersinia enterocolitica* infection." Acta Rheumatol. Scand. **15**(3): 232-253.

Alanis, A. J. (2005). "Resistance to antibiotics: are we in the post-antibiotic era?" Arch. Med. Res. **36**(6): 697-705.

Aleksic, S. (1995). "Occurrence of *Y. enterocolitica* antigens O:3, O:9 and O:8 in different *Yersinia* species, their corresponding H antigens and origin." Contrib. Microbiol. Immunol. **13**: 89-92.

Aleksic, S. and J. Bockemuhl (1987). "Diagnostic importance of H-antigens in *Yersinia enterocolitica* and other *Yersinia* species." Contrib. Microbiol. Immunol. **9**: 279-284.

Aleksic, S., J. Bockemuhl and F. Lange (1986). "Studies on the serology of flagellar antigens of *Yersinia enterocolitica* and related *Yersinia* species." Zentralbl Bakteriol Mikrobiol Hyg [A] **261**(3): 299-310.

Alsterlund, R., M. L. Danielsson-Tham, G. Karp, T. Eden, B. De Jong, P. O. Nilsson and U. Ransjo (1995). "[An outbreak of *Yersinia enterocolitica* infection on the Bjarred peninsula. Indications for risks of refrigerated food]." Lakartidningen **92**(12): 1213-1214.

Anonymous (2009). OzFoodNet Quarterly Report, 1 July to 30 September. OzFoodNet **33**.

Anonymous. (2011). "Statistik för yersiniainfektion, 2010." Retrieved 12th July, 2011, from http://www.smittskyddsinstitutet.se/statistik/yersiniainfektion/?t=com - statistics-nav.

Antikainen, J., A. Kantele, S. H. Pakkanen, T. Laaveri, J. Riutta, M. Vaara and J. Kirveskari (2013). "A quantitative polymerase chain reaction assay for rapid detection of 9 pathogens directly from stools of travelers with diarrhea." Clin Gastroenterol Hepatol **11**(10): 1300-1307 e1303.

Antonopoulos, P., F. Constantinidis, G. Charalampopoulos, K. Dalamarinis, I. Karanicas and G. Kokkini (2008). "An emergency diagnostic dilemma: a case of *Yersinia enterocolitica* colitis mimicking acute appendicitis in a beta-thalassemia major patient: the role of CT and literature review." Emerg Radiol **15**(2): 123-126.

Arbeit, R. D., M. Arthur, R. Dunn, C. Kim, R. K. Selander and R. Goldstein (1990). "Resolution of recent evolutionary divergence among *Escherichia coli* from related lineages: the application of pulsed field electrophoresis to molecular epidemiology." J. Infect. Dis. **161**(2): 230-235.

Archer, J. R., R. F. Schell, D. R. Pennell and P. D. Wick (1987). "Identification of *Yersinia* spp. with the API 20E system." J. Clin. Microbiol. **25**(12): 2398-2399.

Arnold, T., H. Neubauer, M. Ganter, K. Nikolaou, U. Roesler, U. Truyen and A. Hensel (2006). "Prevalence of *Yersinia enterocolitica* in goat herds from Northern Germany." J Vet Med B Infect Dis Vet Public Health **53**(8): 382-386.

Asplund, K., M. Hakkinen, T. Okkonen, P. Vanhala and E. Nurmi (1998). "Effects of growth-promoting antimicrobials on inhibition of *Yersinia enterocolitica* O:3 by porcine ileal microflora." J. Appl. Microbiol. **85**(1): 164-170.

Asplund, K., T. Johansson and A. Siitonen (1998). "Evaluation of pulsed-field gel electrophoresis of genomic restriction fragments in the discrimination of *Yersinia enterocolitica* O:3." Epidemiol. Infect. **121**(3): 579-586.

Ayyadurai, S., C. Flaudrops, D. Raoult and M. Drancourt (2010). "Rapid identification and typing of *Yersinia pestis* and other *Yersinia* species by matrix-assisted laser desorption/ ionization time-of-flight (MALDI-TOF) mass spectrometry." BMC Microbiol **10**: 285.

Babic-Erceg, A., Z. Klismanic, M. Erceg, D. Tandara and M. Smoljanovic (2003). "An outbreak of *Yersinia enterocolitica* O:3 infections on an oil tanker." Eur. J. Epidemiol. **18**(12): 1159-1161.

Barrett, N. J. (1986). "Communicable disease associated with milk and dairy products in England and Wales: 1983-1984." J. Infect. **12**(3): 265-272.

Batzilla, J., U. Antonenka, D. Hoper, J. Heesemann and A. Rakin (2011). "*Yersinia enterocolitica palearctica* serobiotype O:3/4 - a successful group of emerging zoonotic pathogens." BMC Genomics **12**(1): 348.

Batzilla, J., J. Heesemann and A. Rakin (2011). "The pathogenic potential of *Yersinia enterocolitica* 1A." Int. J. Med. Microbiol. **301**(7): 556-571.

Baumgartner, A., M. Kuffer, D. Suter, T. Jemmi and P. Rohner (2007). "Antimicrobial resistance of *Yersinia enterocolitica* strains from human patients, pigs and retail pork in Switzerland." Int. J. Food Microbiol. **115**(1): 110-114.

Benavides, S., K. Nicol, K. Koranyi and M. C. Nahata (2003). "*Yersinia* septic shock following an autologous transfusion in a pediatric patient." Transfus Apher Sci **28**(1): 19-23.

Bhaduri, S., I. Wesley, H. Richards, A. Draughon and M. Wallace (2009). "Clonality and antibiotic susceptibility of *Yersinia enterocolitica* isolated from u.s. market weight hogs." Foodborne Pathog Dis **6**(3): 351-356.

Black, R. E., R. J. Jackson, T. Tsai, M. Medvesky, M. Shayegani, J. C. Feeley, K. I. MacLeod and A. M. Wakelee (1978). "Epidemic *Yersinia enterocolitica* infection due to contaminated chocolate milk." N. Engl. J. Med. **298**(2): 76-79.

Bohr, J., R. Nordfelth, G. Jarnerot and C. Tysk (2002). "*Yersinia* species in collagenous colitis: a serologic study." Scand. J. Gastroenterol. **37**(6): 711-714.

Bonke, R., S. Wacheck, E. Stuber, C. Meyer, E. Martlbauer and M. Fredriksson-Ahomaa (2011). "Antimicrobial susceptibility and distribution of beta-lactamase A (*blaA*) and beta-lactamase B (*blaB*) genes in enteropathogenic *Yersinia* species." Microb Drug Resist **17**(4): 575-581.

Boqvist, S., H. Pettersson, A. Svensson and Y. Andersson (2008). "Sources of sporadic *Yersinia enterocolitica* infection in children in Sweden, 2004: a case-control study." Epidemiol. Infect.: 1-9.

Bottone, E. J. (1997). "*Yersinia enterocolitica*: the charisma continues." Clin. Microbiol. Rev. **10**(2): 257-276.

Bottone, E. J. (1999). "*Yersinia enterocolitica*: overview and epidemiologic correlates." Microbes Infect **1**(4): 323-333.

Bowness, P. (2002). "HLA B27 in health and disease: A double-edged sword?" Rheumatology (Oxford). **41**: 857-868.

Brecher, M. E. and S. N. Hay (2005). "Bacterial contamination of blood components." Clin. Microbiol. Rev. **18**(1): 195-204.

Bresolin, G., J. A. Morgan, D. Ilgen, S. Scherer and T. M. Fuchs (2006). "Low temperature-induced insecticidal activity of *Yersinia enterocolitica*." Mol. Microbiol. **59**(2): 503-512.

Bucher, M., C. Meyer, B. Grotzbach, S. Wacheck, A. Stolle and M. Fredriksson-Ahomaa (2008). "Epidemiological data on pathogenic *Yersinia enterocolitica* in Southern Germany during 2000-2006." Foodborne Pathog Dis **5**(3): 273-280.

Buchrieser, C., S. D. Weagant and C. W. Kaspar (1994). "Molecular characterization of Y*ersinia enterocolitica* by pulsed-field gel electrophoresis and hybridization of DNA fragments to ail and pYV probes." Appl. Environ. Microbiol. **60**(12): 4371-4379.

Burnens, A. P., A. Frey and J. Nicolet (1996). "Association between clinical presentation, biogroups and virulence attributes of *Yersinia enterocolitica* strains in human diarrhoeal disease." Epidemiol. Infect. **116**(1): 27-34.

Butt, H. L., D. L. Gordon, T. Lee-Archer, A. Moritz and W. H. Merrell (1991). "Relationship between clinical and milk isolates of *Yersinia enterocolitica*." Pathology (Phila). **23**(2): 153-157.

Capilla, S., P. Goni, M. C. Rubio, J. Castillo, L. Millan, P. Cerda, J. Sahagun, C. Pitart, A. Beltran and R. Gomez-Lus (2003). "Epidemiological study of resistance to nalidixic acid and other antibiotics in clinical *Yersinia enterocolitica* O:3 isolates." J. Clin. Microbiol. **41**(10): 4876-4878.

Carniel, E. (1999). "The *Yersinia* high-pathogenicity island." Int Microbiol **2**(3): 161-167.

Carter, P. B. (1975). "Pathogenecity of *Yersinia enterocolitica* for mice." Infect. Immun. **11**(1): 164-170.

Chen, P. E., C. Cook, A. C. Stewart, N. Nagarajan, D. D. Sommer, M. Pop, B. Thomason, M. P. Thomason, S. Lentz, N. Nolan, S. Sozhamannan, A. Sulakvelidze, A. Mateczun, L. Du, M. E. Zwick and T. D. Read (2010). "Genomic characterization of the *Yersinia* genus." Genome Biol **11**(1): R1.

Cohen, M. B. (1991). "Etiology and mechanisms of acute infectious diarrhea in infants in the United States." The Journal of Pediatrics **118**(4): 34-39.

Cornelis, G., Y. Laroche, G. Balligand, M. P. Sory and G. Wauters (1987). "*Yersinia enterocolitica*, a primary model for bacterial invasiveness." Rev. Infect. Dis. **9**(1): 64-87.

Cornelis, G. R. and H. Wolf-Watz (1997). "The *Yersinia* Yop virulon: a bacterial system for subverting eukaryotic cells." Mol. Microbiol. **23**(5): 861-867.

Cover, T. L. and R. C. Aber (1989). "*Yersinia enterocolitica*." N. Engl. J. Med. **321**(1): 16-24.

Crowley, E., P. Bird, K. Fisher, K. Goetz, M. Boyle, M. J. Benzinger, Jr., M. Juenger, J. Agin, D. Goins and R. Johnson (2012). "Evaluation of the VITEK 2 Gram-negative (GN) microbial identification test card: collaborative study." J. AOAC Int. **95**(3): 778-785.

de Benito, I., M. E. Cano, J. Aguero and J. M. Garcia Lobo (2004). "A polymorphic tandem repeat potentially useful for typing in the chromosome of *Yersinia enterocolitica*." Microbiology **150**(Pt 1): 199-204.

De Berardis, B., G. Torresini, M. Brucchi, S. Marinelli, S. Mattucci, M. Schietroma, L. Vecchio and F. Carlei (2004). "*Yersinia enterocolitica* intestinal infection with ileum perforation: report of a clinical observation." Acta Biomed **75**(1): 77-81.

de Koning-Ward, T. F. and R. M. Robins-Browne (1995). "Contribution of urease to acid tolerance in *Yersinia enterocolitica*." Infect. Immun. **63**(10): 3790-3795.

de Koning-Ward, T. F., A. C. Ward, E. L. Hartland and R. M. Robins-Browne (1995). "The urease complex gene of *Yersinia enterocolitica* and its role in virulence." Contrib. Microbiol. Immunol. **13**: 262-263.

deGrace, M., M. F. Laurin, C. Belanger, P. E. Rolland, R. Blais, J. P. Breton and G. Martineau (1976). "*Yersinia enterocolitica* gastroenteritis outbreak-Montreal." Can Dis Weekly Rep **2**: 41-42.

Delor, I. and G. R. Cornelis (1992). "Role of *Yersinia enterocolitica* Yst toxin in experimental infection of young rabbits." Infect. Immun. **60**(10): 4269-4277.

Delor, I., A. Kaeckenbeeck, G. Wauters and G. R. Cornelis (1990). "Nucleotide sequence of yst, the *Yersinia enterocolitica* gene encoding the heat-stable enterotoxin, and prevalence of the gene among pathogenic and nonpathogenic *yersiniae*." Infect. Immun. **58**(9): 2983-2988.

Denis, M., E. Houard, A. Labbe, M. Fondrevez and G. Salvat (2011). "A Selective Chromogenic Plate, YECA, for the Detection of Pathogenic *Yersinia enterocolitica*: Specificity, Sensitivity, and Capacity to Detect Pathogenic Y. enterocolitica from Pig Tonsils." J Pathog **2011**: 296275.

Devenish, J. A. and D. A. Schiemann (1981). "An abbreviated scheme for identification of *Yersinia enterocolitica* isolated from food enrichments on CIN (cefsulodin-irgasan-novobiocin) agar." Can. J. Microbiol. **27**(9): 937-941.

Dhar, M. S. and J. S. Virdi (2013). "Interaction of *Yersinia enterocolitica* biovar 1A with cultured cells in vitro does not reflect the two clonal groups identified earlier." J. Med. Microbiol.

Ding, M. J., H. Y. Chiang, C. L. Lin and R. P. Williams (1986). "Isolation and characterization of *Yersinia enterocolitica* in Taipei." Zhonghua Min Guo Wei Sheng Wu Ji Mian Yi Xue Za Zhi **19**(4): 295-301.

Dolina, M. and R. Peduzzi (1993). "Population genetics of human, animal, and environmental *Yersinia* strains." Appl. Environ. Microbiol. **59**(2): 442-450.

Ebringer, R., D. Colthorpe, G. Burden, C. Hindley and A. Ebringer (1982). "*Yersinia enterocolitica* biotype I. Diarrhoea and episodes of HLA B27 related ocular and rheumatic inflammatory disease in South-East England." Scand. J. Rheumatol. **11**(3): 171-176.

EFSA (2005). "EFSA's First Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Antimicrobial resistance in the European Union in 2004. ." The EFSA Journal **310**.

EFSA (2006). "EFSA's Second Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial resistance and Foodborne outbreaks in the European Union in 2005 " The EFSA Journal **94**: 1-236.

EFSA (2010). "The Community Summary Report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in the European Union in 2008 " The EFSA Journal **8**(1): 410.

EFSA (2013). "The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2011." The EFSA Journal **11**(4): 250.

Eitel, J., T. Heise, U. Thiesen and P. Dersch (2005). "Cell invasion and IL-8 production pathways initiated by YadA of *Yersinia pseudotuberculosis* require common signalling molecules (FAK, c-Src, Ras) and distinct cell factors." Cell Microbiol **7**(1): 63-77.

El Tahir, Y. and M. Skurnik (2001). "YadA, the multifaceted *Yersinia* adhesin." Int. J. Med. Microbiol. **291**(3): 209-218.

Ewing, W. H., A. J. Ross, D. J. Brenner and G. R. Fanning (1978). "*Yersinia ruckeri* sp. nov., the Redmouth (RM) Bacterium." Internationa Journal of Systematic Bacteriology **28**(1): 37-44.

Falcao, J. P., D. P. Falcao, A. Pitondo-Silva, A. C. Malaspina and M. Brocchi (2006). "Molecular typing and virulence markers of *Yersinia enterocolitica* strains from human, animal and food origins isolated between 1968 and 2000 in Brazil." J. Med. Microbiol. **55**(Pt 11): 1539-1548.

Farmer, J. J., 3rd, G. P. Carter, V. L. Miller, S. Falkow and I. K. Wachsmuth (1992). "Pyrazinamidase, CR-MOX agar, salicin fermentation-esculin hydrolysis, and D-xylose fermentation for identifying pathogenic serotypes of *Yersinia enterocolitica*." J. Clin. Microbiol. **30**(10): 2589-2594.

Foberg, U., A. Fryden, E. Kihlstrom, K. Persson and O. Weiland (1986). "*Yersinia enterocolitica* septicemia: clinical and microbiological aspects." Scand. J. Infect. Dis. **18**(4): 269-279.

Fredriksson-Ahomaa, M., T. Autio and H. Korkeala (1999). "Efficient subtyping of *Yersinia enterocolitica* bioserotype 4/O:3 with pulsed-field gel electrophoresis." Lett. Appl. Microbiol. **29**(5): 308-312.

Fredriksson-Ahomaa, M., S. Hallanvuo, T. Korte, A. Siitonen and H. Korkeala (2001). "Correspondence of genotypes of sporadic *Yersinia enterocolitica* bioserotype 4/O:3 strains from human and porcine sources." Epidemiol. Infect. **127**(1): 37-47.

Fredriksson-Ahomaa, M., B. Hartmann, P. Scheu and A. Stolle (2007). "Detection of Pathogenic *Yersinia enterocolitica* in Meat using Real-Time PCR." Journal für Verbraucherschutz und Lebensmittelsicherheit **2**(2): 202-208.

Fredriksson-Ahomaa, M. and H. Korkeala (2003). "Molecular epidemiology of *Yersinia enterocolitica* 4/O:3." Adv. Exp. Med. Biol. **529**: 295-302.

Fredriksson-Ahomaa, M., A. Stolle and H. Korkeala (2006). "Molecular epidemiology of *Yersinia enterocolitica* infections." FEMS Immunol. Med. Microbiol. **47**(3): 315-329.

Fredriksson-Ahomaa, M., A. Stolle, A. Siitonen and H. Korkeala (2006). "Sporadic human *Yersinia enterocolitica* infections caused by

bioserotype 4/O : 3 originate mainly from pigs." J. Med. Microbiol. **55**(Pt 6): 747-749.

Fredriksson-Ahomaa, M., A. Stolle and R. Stephan (2007). "Prevalence of pathogenic *Yersinia enterocolitica* in pigs slaughtered at a Swiss abattoir." Int. J. Food Microbiol. **119**(3): 207-212.

Fuchs, T. M., K. Brandt, M. Starke and T. Rattei (2011). "Shotgun sequencing of *Yersinia enterocolitica* strain W22703 (biotype 2, serotype O:9): genomic evidence for oscillation between invertebrates and mammals." BMC Genomics **12**: 168.

Fukushima, H., K. Katsube, Y. Hata, R. Kishi and S. Fujiwara (2007). "Rapid separation and concentration of food-borne pathogens in food samples prior to quantification by viable-cell counting and real-time PCR." Appl. Environ. Microbiol. **73**(1): 92-100.

Galindo, C. L., J. A. Rosenzweig, M. L. Kirtley and A. K. Chopra (2011). "Pathogenesis of *Y. enterocolitic*a and *Y. pseudotuberculosis* in Human Yersiniosis." J Pathog **2011**: 182051.

Garzetti, D., J. Heesemann and A. Rakin (2013). "Genome Sequences of Four *Yersinia enterocolitica* Bioserotype 4/O:3 Isolates from Mammals." Genome Announc **1**(4).

Gemski, P., J. R. Lazere and T. Casey (1980). "Plasmid associated with pathogenicity and calcium dependency of *Yersinia enterocolitica*." Infect. Immun. **27**(2): 682-685.

Gierczyński, R., A. Golubov, H. Neubauer, J. N. Pham and A. Rakin (2007). "Development of multiple-locus variable-number tandem-repeat analysis for *Yersinia enterocolitica* subsp. *palearctica* and its application to bioserogroup 4/O3 subtyping." J. Clin. Microbiol. **45**(8): 2508-2515.

Gort, A. S. and V. L. Miller (2000). "Identification and characterization of *Yersinia enterocolitica* genes induced during systemic infection." Infect. Immun. **68**(12): 6633-6642.

Grahek-Ogden, D., B. Schimmer, K. S. Cudjoe, K. Nygård and G. Kapperud (2007). "Outbreak of *Yersinia enterocolitica* serogroup O:9 infection and processed pork, Norway." Emerg. Infect. Dis. **13**: 754-756.

Granfors, K., M. Ogasawara, J. L. Hill, R. Lahesmaa-Rantala, A. Toivanen and D. T. Yu (1989). "Analysis of IgA antibodies to lipopolysaccharide in *Yersinia*-triggered reactive arthritis." J. Infect. Dis. **159**(6): 1142-1147.

Grant, T., V. Bennett-Wood and R. M. Robins-Browne (1999). "Characterization of the interaction between *Yersinia enterocolitica* biotype 1A and phagocytes and epithelial cells in vitro." Infect. Immun. **67**(9): 4367-4375.

Granum, P. E. (2006). Bacterial toxins as foodpoisons. The Comprehensive Sourcebook of Bacterial Protein Toxins. J. E.

Alouf and M. R. Popoff. London, Elsvier Ltd. **Third**.

Greenwood, J. R., S. M. Flanigan, M. J. Pickett and W. J. Martin (1975). "Clinical isolation of *Yersinia enterocolitica*: cold temperature enrichment." J. Clin. Microbiol. **2**(6): 559-560.

Greenwood, M. H. and W. L. Hooper (1990). "Excretion of *Yersinia* spp. associated with consumption of pasteurized milk." Epidemiol. Infect. **104**(3): 345-350.

Guinet, F., E. Carniel and A. Leclercq (2011). "Transfusion-transmitted *Yersinia enterocolitica* sepsis." Clin. Infect. Dis. **53**(6): 583-591.

Gulati, P., R. K. Varshney and J. S. Virdi (2009). "Multilocus variable number tandem repeat analysis as a tool to discern genetic relationships among strains of *Yersinia enterocolitica* biovar 1A." J. Appl. Microbiol.

Gulati, P. and J. S. Virdi (2007). "The rrn locus and *gyrB* genotyping confirm the existence of two clonal groups in strains of *Yersinia enterocolitica* subspecies *palearctica* biovar 1A." Res. Microbiol. **158**(3): 236-243.

Gumaste, P., V. S. Boppana, A. S. Garcha and D. Blair (2012). "Thigh Abscess Caused by *Yersinia enterocolitica* in an Immunocompetent Host." Case Rep Med **2012**: 259475.

Gutman, L. T., E. A. Ottesen, T. J. Quan, P. S. Noce and S. L. Katz (1973). "An inter-familial outbreak of *Yersinia enterocolitica* enteritis." N. Engl. J. Med. **288**(26): 1372-1377.

Hallanvuo, S., M. Skurnik, K. Asplund and A. Siitonen (2002). "Detection of a novel repeated sequence useful for epidemiological typing of pathogenic *Yersinia enterocolitica*." Int. J. Med. Microbiol. **292**(3-4): 215-225.

Haller, J. C., S. Carlson, K. J. Pederson and D. E. Pierson (2000). "A chromosomally encoded type III secretion pathway in *Yersinia enterocolitica* is important in virulence." Mol. Microbiol. **36**(6): 1436-1446.

Hanifian, S. and S. Khani (2012). "Prevalence of virulent *Yersinia enterocolitica* in bulk raw milk and retail cheese in northern-west of Iran." Int. J. Food Microbiol. **155**(1-2): 89-92.

Hannu, T., R. Inman, K. Granfors and M. Leirisalo-Repo (2006). "Reactive arthritis or post-infectious arthritis?" Best Pract Res Clin Rheumatol **20**(3): 419-433.

Harmon, M. C., C. L. Yu and B. Swaminathan (1983). "An Evaluation of Selective Differential Plating Media for the Isolation of *Yersinia enterocolitica* from Experimentally Inoculated Fresh Ground Pork Homogenate." J. Food Sci. **48**(1): 6-9.

Head, C. B., D. A. Whitty and S. Ratnam (1982). "Comparative study of selective media for recovery of *Yersinia enterocolitica*." J. Clin. Microbiol. **16**(4): 615-621.

Heesemann, J., B. Algermissen and R. Laufs (1984). "Genetically manipulated virulence of *Yersinia enterocolitica*." Infect. Immun. **46**(1): 105-110.

Heesemann, J. and R. Laufs (1983). "Construction of a mobilizable *Yersinia enterocolitica* virulence plasmid." J. Bacteriol. **155**(2): 761-767.

Helms, M., J. Simonsen and K. Molbak (2006). "Foodborne bacterial infection and hospitalization: a registry-based study." Clin. Infect. Dis. **42**(4): 498-506.

Heusipp, G., G. M. Young and V. L. Miller (2001). "HreP, an in vivo-expressed protease of *Yersinia enterocolitica*, is a new member of the family of subtilisin/kexin-like proteases." J. Bacteriol. **183**(12): 3556-3563.

Hoogkamp-Korstanje, J. A. and V. M. Stolk-Engelaar (1995). "*Yersinia enterocolitica* infection in children." Pediatr. Infect. Dis. J. **14**(9): 771-775.

Howard, S. L., M. W. Gaunt, J. Hinds, A. A. Witney, R. Stabler and B. W. Wren (2006). "Application of comparative phylogenomics to study the evolution of *Yersinia enterocolitica* and to identify genetic differences relating to pathogenicity." J. Bacteriol. **188**(10): 3645-3653.

Hrycaj, P. and J. K. Lacki (2003). "Antibodies to ubiquitin in relation to *Yersinia* infection status in patients with ankylosing spondylitis." Clin. Rheumatol. **22**(1): 33-36.

Huovinen, E. and L. Sihvonen (2011). *Yersinia*. Infectious Diseases in Finland 2010. T. e. a. Hulkko. Helsinki, National Institute for Health and Welfare, Finland. **39/2011:** 64.

Hurst, M. R., S. A. Becher, S. D. Young, T. L. Nelson and T. R. Glare (2010). "*Yersinia entomophaga* sp. nov. isolated from the New Zealand grass grub *Costelytra zealandica*." Int J Syst Evol Microbiol.

Ibrahim, A., B. M. Goebel, W. Liesack, M. Griffiths and E. Stackebrandt (1993). "The phylogeny of the genus *Yersinia* based on 16S rDNA sequences." FEMS Microbiol. Lett. **114**(2): 173-177.

Ibrahim, A., W. Liesack and E. Stackebrandt (1992). "Polymerase chain reaction-gene probe detection system specific for pathogenic strains of *Yersinia enterocolitica*." J. Clin. Microbiol. **30**(8): 1942-1947.

Ibrahim, A., W. Liesack, A. G. Steigerwalt, D. J. Brenner, E. Stackebrandt and R. M. Robins-Browne (1997). "A cluster of atypical *Yersinia* strains with a distinctive 16S rRNA signature." FEMS Microbiol. Lett. **146**(1): 73-78.

Iriarte, M. and G. R. Cornelis (1995). "MyfF, an element of the network regulating the synthesis of fibrillae in *Yersinia enterocolitica*." J. Bacteriol. **177**(3): 738-744.

Iteman, I., A. Guiyoule and E. Carniel (1996). "Comparison of three molecular methods for typing and subtyping pathogenic *Yersinia enterocolitica* strains." J. Med. Microbiol. **45**(1): 48-56.

Iwata, T., Y. Une, A. T. Okatani, S. Kaneko, S. Namai, S. Yoshida, T. Horisaka, T. Horikita, A. Nakadai and H. Hayashidani (2005). "*Yersinia enterocolitica* serovar O:8 infection in breeding monkeys in Japan." Microbiol. Immunol. **49**(1): 1-7.

Iwobi, A., J. Heesemann, E. Garcia, E. Igwe, C. Noelting and A. Rakin (2003). "Novel virulence-associated type II secretion system unique to high-pathogenicity *Yersinia enterocolitica*." Infect. Immun. **71**(4): 1872-1879.

Janda, J. M. and S. L. Abbott (2010). "The genus *Aeromonas*: taxonomy, pathogenicity, and infection." Clin. Microbiol. Rev. **23**(1): 35-73.

Jones, T. F. (2003). "From pig to pacifier: chitterling-associated yersiniosis outbreak among black infants." Emerg. Infect. Dis. **9**(8): 1007-1009.

Kahlau, P., M. Malecki, V. Schildgen, C. Schulz, I. Winterfeld, S. Messler, F. Mattner and O. Schildgen (2013). "Utility of two novel multiplexing assays for the detection of gastrointestinal pathogens - a first experience." Springerplus **2**(1): 106.

Kanamori, M. (1976). "Biological activities of endotoxins from *Yersinia enterocolitica*." Jpn. J. Microbiol. **20**(4): 273-280.

Kapperud, G. (1991). "*Yersinia enterocolitica* in food hygiene." Int. J. Food Microbiol. **12**(1): 53-65.

Karhukorpi, J. and M. Päivänurmi (2013). "Differentiation of *Yersinia enterocolitica* biotype 1A from pathogenic *Yersinia enterocolitica* biotypes by detection of beta-glucosidase activity: Comparison of two chromogenic culture media and Vitek2." J. Med. Microbiol.

Kasatiya, S. (1976). "*Yersinia enterocolitica* gastroenteritis outbreak - Montreal." Can Dis Weekly Rep **2**: 73-74.

Kay, W. W., B. M. Phipps, E. E. Ishiguro and T. J. Trust (1985). "Porphyrin binding by the surface array virulence protein of *Aeromonas salmonicida*." J. Bacteriol. **164**(3): 1332-1336.

Khan, M. A. (1995). "HLA-B27 and its subtypes in world populations." Curr. Opin. Rheumatol. **7**(4): 263-269.

Klinzing, D. C., R. R. Matias, E. Skowronski, M. Alvarez, V. Liles, M. P. Dimamay and F. F. Natividad (2012). "Shotgun genome sequence of a *Yersinia enterocolitica* isolate from the Philippines." J. Bacteriol. **194**(2): 542-543.

Knösel, T., C. Schewe, N. Petersen, M. Dietel and I. Petersen (2009). "Prevalence of infectious pathogens in Crohn's disease." Pathol. Res. Pract. **205**(4): 223-230.

Koehler, K. M., T. Lasky, S. B. Fein, S. M. Delong, M. A. Hawkins, T. Rabatsky-Ehr, S. M. Ray, B. Shiferaw, E. Swanson and D. J. Vugia (2006). "Population-based incidence of infection with selected bacterial enteric pathogens in children younger than five years of age, 1996-

1998." Pediatr. Infect. Dis. J. **25**(2): 129-134.

Kontiainen, S., A. Sivonen and O.-V. Renkonen (1994). "Increased yields of pathogenic *Yersinia enterocolitica* strains by cold enrichment." Scand. J. Infect. Dis. **26**: 685-691.

Korte, T., M. Fredriksson-Ahomaa, T. Niskanen and H. Korkeala (2004). "Low prevalence of yadA-positive *Yersinia enterocolitica* in sows." Foodborne Pathog Dis **1**(1): 45-52.

Kraushaar, B., R. Dieckmann, M. Wittwer, D. Knabner, A. Konietzny, D. Made and E. Strauch (2011). "Characterization of a *Yersinia enterocolitica* biotype 1A strain harbouring an *ail* gene." J. Appl. Microbiol. **111**(4): 997-1005.

Kuehni-Boghenbor, K., S. L. On, B. Kokotovic, A. Baumgartner, T. M. Wassenaar, M. Wittwer, B. Bissig-Choisat and J. Frey (2006). "Genotyping of human and porcine *Yersinia enterocolitica*, Y*ersinia intermedia*, and *Yersinia bercovieri* strains from Switzerland by amplified fragment length polymorphism analysis." Appl. Environ. Microbiol. **72**(6): 4061-4066.

Kuhm, A. E., D. Suter, R. Felleisen and J. Rau (2009). "Application of Fourier Transform Infrared Spectroscopy (FT-IR) for the Identification of *Yersinia enterocolitica* on Species and Subspecies Level." Appl. Environ. Microbiol.

Laitenen, O., J. Tuuhea and P. Ahvonen (1972). "Polyarthritis associated with *Yersinia enterocolitica* infection. Clinical features and laboratory findings in nine cases with severe joint symptoms." Ann. Rheum. Dis. **31**(1): 34-39.

Lambertz, S. T., C. Nilsson and S. Hallanvuo (2008). "TaqMan-based real-time PCR method for detection of *Yersinia pseudotuberculosis* in food." Appl. Environ. Microbiol. **74**(20): 6465-6469.

Lambertz, S. T., C. Nilsson, S. Hallanvuo and M. Lindblad (2008). "Real-time PCR method for detection of pathogenic *Yersinia enterocolitica* in food." Appl. Environ. Microbiol. **74**(19): 6060-6067.

Lanada, E. B., R. S. Morris, R. Jackson and S. G. Fenwick (2005). "A cohort study of *Yersinia* infection in goats." Aust. Vet. J. **83**(9): 567-571.

Lasch, P., M. Drevinek, H. Nattermann, R. Grunow, M. Stammler, R. Dieckmann, T. Schwecke and D. Naumann (2010). "Characterization of *Yersinia* Using MALDI-TOF Mass Spectrometry and Chemometrics." Anal. Chem. **82**(20): 8464-8475.

Leclercq, A., L. Martin, M. L. Vergnes, N. Ounnoughene, J. F. Laran, P. Giraud and E. Carniel (2005). "Fatal *Yersinia enterocolitica* biotype 4 serovar O:3 sepsis after red blood cell transfusion." Transfusion (Paris). **45**(5): 814-818.

Lee, L. A., A. R. Gerber, D. R. Lonsway, J. D. Smith, G. P.

Carter, N. D. Puhr, C. M. Parrish, R. K. Sikes, R. J. Finton and R. V. Tauxe (1990). "*Yersinia enterocolitica* O:3 infections in infants and children, associated with the household preparation of chitterlings." N. Engl. J. Med. **322**(14): 984-987.

Lee, L. A., J. Taylor, G. P. Carter, B. Quinn, J. J. Farmer, 3rd and R. V. Tauxe (1991). "*Yersinia enterocolitica* O:3: an emerging cause of pediatric gastroenteritis in the United States. The Yersinia enterocolitica Collaborative Study Group." J. Infect. Dis. **163**(3): 660-663.

Leirisalo-Repo, M. (2005). "Reactive arthritis." Scand. J. Rheumatol. **34**(4): 251-259.

Linde, H. J., H. Neubauer, H. Meyer, S. Aleksic and N. Lehn (1999). "Identification of *Yersinia* species by the Vitek GNI card." J. Clin. Microbiol. **37**(1): 211-214.

Lindholm, H. and R. Visakorpi (1991). "Late complications after a *Yersinia enterocolitica* epidemic: a follow up study." Ann. Rheum. Dis. **50**(10): 694-696.

Lindstedt, B. A. (2005). "Multiple-locus variable number tandem repeats analysis for genetic fingerprinting of pathogenic bacteria." Electrophoresis **26**(13): 2567-2582.

Lindstedt, B. A. (2011). "Genotyping of selected bacterial enteropathogens in Norway." Int. J. Med. Microbiol. **301**(8): 648-653.

Lobato, M. J., E. Landeras, M. A. Gonzalez-Hevia and M. C. Mendoza (1998). "Genetic heterogeneity of clinical strains of *Yersinia enterocolitica* traced by ribotyping and relationships between ribotypes, serotypes, and biotypes." J. Clin. Microbiol. **36**(11): 3297-3302.

Loftus, C. G., G. C. Harewood, F. R. Cockerill, 3rd and J. A. Murray (2002). "Clinical features of patients with novel *Yersinia* species." Dig. Dis. Sci. **47**(12): 2805-2810.

Määttä, J., M. Lehto, R. Kuisma, H. R. Kymäläinen and M. Mäki (2013). "Microbiological quality of fresh-cut carrots and process waters." J. Food Prot. **76**(7): 1240-1244.

Macdonald, E., B. Heier, T. Stalheim, K. Cudjoe, T. Skjerdal, A. Wester, B. Lindstedt and L. Vold (2011). "*Yersinia enterocolitica* O:9 infections associated with bagged salad mix in Norway, February to April 2011." Euro Surveill **16**(19).

MacDonald, E., B. T. Heier, K. Nygard, T. Stalheim, K. S. Cudjoe, T. Skjerdal, A. L. Wester, B. A. Lindstedt, T. L. Stavnes and L. Vold (2012). "*Yersinia enterocolitica* outbreak associated with ready-to-eat salad mix, Norway, 2011." Emerg. Infect. Dis. **18**(9): 1496-1499.

Mäki-Ikola, O., J. Heesemann, A. Toivanen and K. Granfors (1997). "High frequency of *Yersinia* antibodies in healthy populations in Finland and Germany." Rheumatol. Int. **16**(6): 227-229.

Mäkinen, M., S. Niemela, J. Lehtola and T. J. Karttunen (1998). "Collagenous colitis and

Yersinia enterocolitica infection." Dig. Dis. Sci. **43**(6): 1341-1346.

Marjai, E., M. Kalman, I. Kajary, A. Belteky and M. Rodler (1987). "Isolation from food and characterization by virulence tests of *Yersinia enterocolitica* associated with an outbreak." Acta Microbiol. Hung. **34**(2): 97-109.

Marks, M. I., C. H. Pai, L. Lafleur, L. Lackman and O. Hammerberg (1980). "*Yersinia enterocolitica* gastroenteritis: a prospective study of clinical, bacteriologic, and epidemiologic features." J. Pediatr. **96**(1): 26-31.

Marranzano, M., M. A. Coniglio and L. Mauro (2003). "Evaluation of pulsed-field gel electrophoresis (PFGE) for *Yersinia enterocolitica* molecular epidemiology investigations." Adv. Exp. Med. Biol. **529**: 375-378.

Martin, T., G. F. Kasian and S. Stead (1982). "Family outbreak of yersiniosis." J. Clin. Microbiol. **16**(4): 622-626.

Maruyama, T. (1987). "*Yersinia enterocolitica* infection in humans and isolation of the microorganism from pigs in Japan." Contr Microbiol Immunol **9**: 48-55.

Matsumoto, T., M. Iida, T. Sakai, Y. Kimura and M. Fujishima (1991). "*Yersinia* terminal ileitis: sonographic findings in eight patients." AJR. Am. J. Roentgenol. **156**(5): 965-967.

Mayrhofer, S., P. Paulsen, F. J. Smulders and F. Hilbert (2004). "Antimicrobial resistance profile of five major food-borne pathogens isolated from beef, pork and poultry." Int. J. Food Microbiol. **97**(1): 23-29.

McDowell, R. M. and M. D. McElvaine (1997). "Long-term sequelae to foodborne disease." Rev. Sci. Tech. **16**(2): 337-341.

McIntyre, M. and E. Nnochiri (1986). "A case of hospital-acquired *Yersinia enterocolitica* gastro-enteritis." J. Hosp. Infect. **7**(3): 299-301.

McNally, A. (2007). "An aflagellate mutant *Yersinia enterocolitica* biotype 1A strain displays altered invasion of epithelial cells, persistence in macrophages, and cytokine secretion profiles in vitro." Microbiology **153**(Pt 5): 1339-1388.

McNally, A., T. Cheasty, C. Fearnley, R. W. Dalziel, G. A. Paiba, G. Manning and D. G. Newell (2004). "Comparison of the biotypes of *Yersinia enterocolitica* isolated from pigs, cattle and sheep at slaughter and from humans with yersiniosis in Great Britain during 1999-2000." Lett. Appl. Microbiol. **39**(1): 103-108.

McNally, A., T. Dalton, R. M. L. Ragione, K. Stapleton, G. Manning and D. G. Newell (2006). "*Yersinia enterocolitica* isolates of differing biotypes from humans and animals are adherent, invasive and persist in macrophages, but differ in cytokine secretion profiles in vitro." J. Med. Microbiol. **55**(12): 1725-1734.

Merhej, V., T. Adekambi, I. Pagnier, D. Raoult and M. Drancourt (2008). "*Yersinia massiliensis* sp. nov., isolated from fresh water." Int J Syst Evol Microbiol **58**(Pt 4): 779-784.

Merilahti-Palo, R., R. Lahesmaa, K. Granfors, C. Gripenberg-Lerche and P. Toivanen (1991). "Risk of *Yersinia* infection among butchers." Scand. J. Infect. Dis. **23**(1): 55-61.

Messelhausser, U., P. Kampf, J. Colditz, H. Bauer, H. Schreiner, C. Holler and U. Busch (2011). "Qualitative and quantitative detection of human pathogenic *Yersinia enterocolitica* in different food matrices at retail level in Bavaria." Foodborne Pathog Dis **8**(1): 39-44.

Miller, V. L., K. B. Beer, G. Heusipp, B. M. Young and M. R. Wachtel (2001). "Identification of regions of Ail required for the invasion and serum resistance phenotypes." Mol. Microbiol. **41**(5): 1053-1062.

Miller, V. L. and S. Falkow (1988). "Evidence for two genetic loci in *Yersinia enterocolitica* that can promote invasion of epithelial cells." Infect. Immun. **56**(5): 1242-1248.

Miller, V. L., J. J. Farmer, 3rd, W. E. Hill and S. Falkow (1989). "The ail locus is found uniquely in *Yersinia enterocolitica* serotypes commonly associated with disease." Infect. Immun. **57**(1): 121-131.

Milnes, A. S., I. Stewart, F. A. Clifton-Hadley, R. H. Davies, D. G. Newell, A. R. Sayers, T. Cheasty, C. Cassar, A. Ridley, A. J. C. Cook, S. J. Evans, C. J. Teale, R. P. Smith, N. A. Mc, M. Toszeghy, R. Futter, A. Kay and G. A. Paiba (2008). "Intestinal carriage of verocytotoxigenic *Escherichia coli* O157, *Salmonella*, thermophilic *Campylobacter* and *Yersinia enterocolitica*, in cattle, sheep and pigs at slaughter in Great Britain during 2003." Epidemiol. Infect. **136**(6): 739-751.

Moriki, S., A. Nobata, H. Shibata, A. Nagai, N. Minami, T. Taketani and H. Fukushima (2010). "Familial outbreak of *Yersinia enterocolitica* serotype O9 biotype 2." J Infect Chemother **16**(1): 56-58.

Morris, J. G., Jr., V. Prado, C. Ferreccio, R. M. Robins-Browne, A. M. Bordun, M. Cayazzo, B. A. Kay and M. M. Levine (1991). "*Yersinia enterocolitica* isolated from two cohorts of young children in Santiago, Chile: incidence of and lack of correlation between illness and proposed virulence factors." J. Clin. Microbiol. **29**(12): 2784-2788.

Morse, D. L., M. Shayegani and R. J. Gallo (1984). "Epidemiologic investigation of a *Yersinia* camp outbreak linked to a food handler." Am. J. Public Health **74**: 589-592.

Much, P., J. Pichler and F. Allerberger (2007). "Lebensmittelbedingte infektiöse Krankheitsausbrüche, Österreich 2005." Wien. Klin. Wochenschr. **119**(5-6): 150-157.

Murros-Kontiainen, A. E., M. Fredriksson-Ahomaa, H.

Korkeala, P. Johansson, R. Rahkila and J. Bjorkroth (2010). "*Yersinia nurmii* sp. nov." Int J Syst Evol Microbiol.

Murros-Kontiainen, A. E., P. Johansson, T. Niskanen, M. Fredriksson-Ahomaa, H. Korkeala and J. Bjorkroth (2010). "*Yersinia pekkanenii* sp. nov." Int J Syst Evol Microbiol.

Najdenski, H., E. Golkocheva, A. Vesselinova, J. A. Bengoechea and M. Skurnik (2003). "Proper expression of the O-antigen of lipopoly-saccharide is essential for the virulence of *Yersinia enterocolitica* O:8 in experimental oral infection of rabbits." FEMS Immunol. Med. Microbiol. **38**(2): 97-106.

Najdenski, H., I. Iteman and E. Carniel (1994). "Efficient subtyping of pathogenic *Yersinia enterocolitica* strains by pulsed-field gel electro-phoresis." J. Clin. Microbiol. **32**(12): 2913-2920.

Navidad, J. F., D. J. Griswold, M. S. Gradus and S. Bhattacharyya (2013). "Evaluation of Luminex xTAG Gastrointestinal Pathogen Analyte-Specific Reagents for High-Throughput, Simultaneous Detection of Bacteria, Viruses, and Parasites of Clinical and Public Health Importance." J. Clin. Microbiol. **51**(9): 3018-3024.

Neubauer, H., A. Hensel, S. Aleksic and H. Meyer (2000). "Identification of *Yersinia enterocolitica* within the genus *Yersinia*." Syst. Appl. Microbiol. **23**(1): 58-62.

Neubauer, H., T. Sauer, H. Becker, S. Aleksic and H. Meyer (1998). "Comparison of systems for identification and differentiation of species within the genus *Yersinia*." J. Clin. Microbiol. **36**(11): 3366-3368.

Niskanen, T. (2007). *Yersinia enterocolitica*, *Yersinia pseudotuberculos* and apatogenic *Yersinia*-species in domestic vegetable [In Finnish]. Project report. Helsinki, Evira**:** 17p.

Noble, M. A., R. L. Barteluk, H. J. Freeman, R. Subramaniam and J. B. Hudson (1987). "Clinical significance of virulence-related assay of *Yersinia* species." J. Clin. Microbiol. **25**(5): 802-807.

Olsovsky, Z., V. Olsakova, S. Chobot and V. Sviridov (1975). "Mass occurrence of *Yersinia enterocolitica* in two establishments of collective care of children." J. Hyg. Epidemiol. Microbiol. Immunol. **19**(1): 22-29.

Paerregaard, A., F. Espersen and M. Skurnik (1991). "Role of the *Yersinia* outer membrane protein YadA in adhesion to rabbit intestinal tissue and rabbit intestinal brush border membrane vesicles." APMIS **99**(3): 226-232.

Pai, C. H., S. Sorger, L. Lafleur, L. Lackman and M. I. Marks (1979). "Efficacy of cold enrichment techniques for recovery of *Yersinia enterocolitica* from human stools." J. Clin. Microbiol. **9**(6): 712-715.

Palonen, E., M. Lindstrom and H. Korkeala (2010). "Adaptation

of enteropathogenic *Yersinia* to low growth temperature." Crit. Rev. Microbiol. **36**(1): 54-67.

Pelludat, C., A. Rakin, C. A. Jacobi, S. Schubert and J. Heesemann (1998). "The yersiniabactin biosynthetic gene cluster of *Yersinia enterocolitica*: organization and siderophore-dependent regulation." J. Bacteriol. **180**(3): 538-546.

Pepe, J. C. and V. L. Miller (1993). "The biological role of invasin during a *Yersinia enterocolitica* infection." Infect. Agents Dis. **2**(4): 236-241.

Pepe, J. C. and V. L. Miller (1993). "*Yersinia enterocolitica* invasin: a primary role in the initiation of infection." Proc. Natl. Acad. Sci. U. S. A. **90**(14): 6473-6477.

Perdikogianni, C., E. Galanakis, M. Michalakis, E. Giannoussi, S. Maraki, Y. Tselentis and G. Charissis (2006). "*Yersinia enterocolitica* infection mimicking surgical conditions." Pediatr. Surg. Int. **22**(7): 589-592.

Petersel, D. L. and L. H. Sigal (2005). "Reactive arthritis." Infect. Dis. Clin. North Am. **19**(4): 863-883.

Pham, J. N., S. M. Bell and J. Y. Lanzarone (1991). "A study of the beta-lactamases of 100 clinical isolates of *Yersinia enterocolitica*." J. Antimicrob. Chemother. **28**(1): 19-24.

Pham, J. N., S. M. Bell, L. Martin and E. Carniel (2000). "The beta-lactamases and beta-lactam antibiotic susceptibility of *Yersinia enterocolitica*." J. Antimicrob. Chemother. **46**(6): 951-957.

Pierson, D. E. and S. Falkow (1990). "Nonpathogenic isolates of *Yersinia enterocolitica* do not contain functional inv-homologous sequences." Infect. Immun. **58**(4): 1059-1064.

Pierson, D. E. and S. Falkow (1993). "The ail gene of *Yersinia enterocolitica* has a role in the ability of the organism to survive serum killing." Infect. Immun. **61**(5): 1846-1852.

Portnoy, D. A., S. L. Moseley and S. Falkow (1981). "Characterization of plasmids and plasmid-associated determinants of *Yersinia enterocolitica* pathogenesis." Infect. Immun. **31**(2): 775-782.

Prats, G., B. Mirelis, T. Llovet, C. Munoz, E. Miro and F. Navarro (2000). "Antibiotic resistance trends in entero-pathogenic bacteria isolated in 1985-1987 and 1995-1998 in Barcelona." Antimicrob. Agents Chemother. **44**(5): 1140-1145.

Prpic, J. K., R. M. Robins-Browne and R. B. Davey (1983). "Differentiation between virulent and avirulent *Yersinia enterocolitica* isolates by using Congo red agar." J. Clin. Microbiol. **18**(3): 486-490.

Rasmussen, H. N., J. E. Olsen and O. F. Rasmussen (1994). "RAPD analysis of *Yersinia enterocolitica*." Lett. Appl. Microbiol. **19**(5): 359-362.

Ratnam, S., E. Mercer, B. Picco, S. Parsons and R. Butler (1982). "A nosocomial out-break of diarrheal disease due to *Yersinia enterocolitica* serotype 0:5, biotype 1." J. Infect. Dis. **145**(2): 242-247.

Renaud, N., L. Lecci, R. J. Courcol, M. Simonet and O. Gaillot (2013). "CHROMagar Yersinia, a new chromogenic agar for screening of potentially pathogenic *Yersinia enterocolitica* isolates in stools." J. Clin. Microbiol. **51**(4): 1184-1187.

Reuter, S., N. R. Thomson and A. McNally (2012). "Evolutionary dynamics of the *Yersinia enterocolitica* complex." Adv. Exp. Med. Biol. **954**: 15-22.

Riley, G. and S. Toma (1989). "Detection of pathogenic *Yersinia enterocolitica* by using congo red-magnesium oxalate agar medium." J. Clin. Microbiol. **27**(1): 213-214.

Rimhanen-Finne, R., T. Niskanen, S. Hallanvuo, P. Makary, K. Haukka, S. Pajunen, A. Siitonen, R. Ristolainen, H. Poyry, J. Ollgren and M. Kuusi (2009). "*Yersinia pseudotuberculosis* causing a large outbreak associated with carrots in Finland, 2006." Epidemiol. Infect. **137**(3): 342-347.

Rizzardi, K., T. Wahab and C. Jernberg (2013). "Rapid subtyping of *Yersinia enterocolitica* by MALDI-TOF MS for diagnostics and surveillance." J. Clin. Microbiol.

Robbins-Browne, R. (2007). *Yersinia enterocolitica*. Food Microbiology: Fundamentals and Frontiers. M. Doyle and L. R. Beuchat. Washington, D.C., ASM Press**:** 293-322.

Robins-Browne, R. M. (1989). "Evaluation of DNA colony hybridization and other techniques for detection of virulence in *Yersinia* species." J. Clin. Microbiol. **27**(4): 644-694.

Robins-Browne, R. M. (2007). *Yersinia enterocolitica*. Food Microbiology: Fundamentals and Frontiers. Washington, D C, ASM Press.

Rose, F. B., C. J. Camp and E. J. Antes (1987). "Family outbreak of fatal *Yersinia enterocolitica* pharyngitis." Am. J. Med. **82**(3 Spec No): 636-637.

Rosner, B. M., K. Stark, M. Hohle and D. Werber (2012). "Risk factors for sporadic *Yersinia enterocolitica* infections, Germany 2009-2010." Epidemiol. Infect. **140**(10): 1738-1747.

Rosner, B. M., K. Stark and D. Werber (2010). "Epidemiology of reported *Yersinia enterocolitica* infections in Germany, 2001-2008." BMC Public Health **10**: 337.

Rosner, B. M., D. Werber, M. Hohle and K. Stark (2013). "Clinical aspects and self-reported symptoms of sequelae of *Yersinia enterocolitica* infections in a population-based study, Germany 2009-2010." BMC Infect Dis **13**: 236.

Sachdeva, P. and J. S. Virdi (2004). "Repetitive elements sequence (REP/ERIC)-PCR based genotyping of clinical and environmental strains of *Yersinia enterocolitica* biotype 1A reveal existence of limited number of clonal groups." FEMS Microbiol. Lett. **240**(2): 193-201.

Saebo, A., E. Vik, O. J. Lange and L. Matuszkiewicz (2005). "Inflammatory bowel disease

associated with *Yersinia enterocolitica* O:3 infection." Eur J Intern Med **16**(3): 176-182.

Sagel, U. and S. Pekard-Amenitsch (2011). Nationale Referenz-zentrale für Yersinien - Jahresbericht 2010. BMG Newsletter Öffentliche Gesundheit.

Sakai, T., A. Nakayama, M. Hashida, Y. Yamamoto, H. Takebe and S. Imai (2005). "Outbreak of food poisoning by *Yersinia enterocolitica* serotype O8 in Nara prefecture: the first case report in Japan." Jpn J Infect Dis **58**(4): 257-258.

Saken, E., A. Roggenkamp, S. Aleksic and J. Heesemann (1994). "Characterisation of pathogenic *Yersinia entero-colitica* serogroups by pulsed-field gel electrophoresis of genomic *Not*I restriction fragments." J. Med. Microbiol. **41**(5): 329-338.

Sanchez-Cespedes, J., M. M. Navia, R. Martinez, B. Orden, R. Millan, J. Ruiz and J. Vila (2003). "Clonal dissemination of *Yersinia enterocolitica* strains with various susceptibilities to nalidixic acid." J. Clin. Microbiol. **41**(4): 1769-1771.

Satterthwaite, P., K. Pritchard, D. Floyd and B. Law (1999). "A case-control study of *Yersinia enterocolitica* infections in Auckland." Aust. N. Z. J. Public Health **23**(5): 482-485.

Savin, C., L. Frangeul, L. Ma, C. Bouchier, I. Moszer and E. Carniel (2013). "Draft genome gequence of a clinical strain of *Yersinia enterocolitica* (IP10393) of bioserotype 4/O:3 from France." Genome Announc **1**(1).

Schiemann, D. A. (1979). "Synthesis of a selective agar medium for *Yersinia enterocolitica*." Can. J. Microbiol. **25**(11): 1298-1304.

Schmid, Y., G. A. Grassl, O. T. Buhler, M. Skurnik, I. B. Autenrieth and E. Bohn (2004). "*Yersinia enterocolitica* adhesin A induces production of interleukin-8 in epithelial cells." Infect. Immun. **72**(12): 6780-6789.

Schwartz, D. C. and C. R. Cantor (1984). "Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis." Cell **37**(1): 67-75.

Shayegani, M., D. Morse, I. DeForge, T. Root, L. M. Parsons and P. S. Maupin (1983). "Microbiology of a major foodborne outbreak of gastroenteritis caused by *Yersinia enterocolitica* sero-group O:8." J. Clin. Microbiol. **17**(1): 35-40.

Shmilovitz, M. and B. Kretzer (1978). "Isolates of *Yersinia entero colitica* from clinical cases in Northern Israel." Isr. J. Med. Sci. **14**(10): 1048-1055.

Skurnik, M. (2003). "Molecular genetics, biochemistry and biological role of *Yersinia* lipopolysaccharide." Adv. Exp. Med. Biol. **529**: 187-197.

Skurnik, M., T. Nurmi, K. Granfors, M. Koskela and A. S. Tiilikainen (1983). "Plasmid associated antibody production against *Yersinia enterocolitica*

in man." Scand. J. Infect. Dis. **15**(2): 173-177.

Skurnik, M. and P. Toivanen (1993). "*Yersinia enterocolitica* lipopolysaccharide: genetics and virulence." Trends Microbiol. **1**(4): 148-152.

Skurnik, M., R. Venho, J. A. Bengoechea and I. Moriyon (1999). "The lipopolysaccharide outer core of *Yersinia enterocolitica* serotype O:3 is required for virulence and plays a role in outer membrane integrity." Mol. Microbiol. **31**(5): 1443-1462.

Soderqvist, K., S. Boqvist, G. Wauters, I. Vagsholm and S. Thisted-Lambertz (2012). "*Yersinia enterocolitica* in sheep--a high frequency of biotype 1A." Acta Vet. Scand. **54**: 39.

Sonnevend, A., E. Czirok and T. Pal (2005). "*Yersinia* Yop-specific IgA antibodies in Hungarian blood donors." Folia Microbiol. (Praha). **50**(3): 269-272.

Souza, R. A., P. F. Imori and J. P. Falcao (2013). "Multilocus sequence analysis and 16S rRNA gene sequencing reveal that *Yersinia frederiksenii* genospecies 2 is Yersinia massiliensis." Int J Syst Evol Microbiol **63**(Pt 8): 3124-3129.

Sprague, L. D. and H. Neubauer (2005). "*Yersinia aleksiciae* sp. nov." Int J Syst Evol Microbiol **55**(Pt 2): 831-835.

Sprague, L. D., H. C. Scholz, S. Amann, H. J. Busse and H. Neubauer (2008). "*Yersinia similis* sp. nov." Int J Syst Evol Microbiol **58**(Pt 4): 952-958.

Stephan, R., N. Cernela, D. Ziegler, V. Pfluger, M. Tonolla, D. Ravasi, M. Fredriksson-Ahomaa and H. Hachler (2011). "Rapid species specific identification and subtyping of *Yersinia enterocolitica* by MALDI-TOF mass spectrometry." J. Microbiol. Methods **87**(2): 150-153.

Stock, I., P. Heisig and B. Wiedemann (1999). "Expression of beta-lactamases in *Yersinia enterocolitica* strains of biovars 2, 4 and 5." J. Med. Microbiol. **48**(11): 1023-1027.

Sulakvelidze, A., A. Kreger, A. Joseph, R. M. Robins-Browne, A. Fasano, G. Wauters, N. Harnett, L. DeTolla and J. G. Morris, Jr. (1999). "Production of enterotoxin by *Yersinia bercovieri*, a recently identified *Yersinia entero-colitica*-like species." Infect. Immun. **67**(2): 968-971.

Tacket, C. O., J. Ballard, N. Harris, J. Allard, C. Nolan, T. Quan and M. L. Cohen (1985). "An outbreak of *Yersinia enterocolitica* infections caused by contaminated tofu (soybean curd)." Am. J. Epidemiol. **121**(5): 705-711.

Tacket, C. O., B. R. Davis, G. P. Carter, J. F. Randolph and M. L. Cohen (1983). "*Yersinia enterocolitica* pharyngitis." Ann. Intern. Med. **99**(1): 40-42.

Tacket, C. O., J. P. Narain, R. Sattin, J. P. Lofgren, C. Konigsberg Jr, R. C. Rendtorff, A. Rausa, B. R. Davis and M. L. Cohen (1984). "A multistate outbreak of infections caused

by *Yersinia enterocolitica* transmitted by pasteurized milk." JAMA **251**: 483-486.

Tafjord Heier, B., K. Nygård and J. Lassen (2007) "Yersiniose i Norge 2006."

Tauxe, R. V., J. Vandepitte, G. Wauters, S. M. Martin, V. Goossens, P. De Mol, R. Van Noyen and G. Thiers (1987). "*Yersinia enterocolitica* infections and pork: the missing link." Lancet **1**(8542): 1129-1132.

Tennant, S. M., E. L. Hartland, T. Phumoonna, D. Lyras, J. I. Rood, R. M. Robins-Browne and I. R. van Driel (2008). "Influence of gastric acid on susceptibility to infection with ingested bacterial pathogens." Infect. Immun. **76**(2): 639-645.

Tennant, S. M., N. A. Skinner, A. Joe and R. M. Robins-Browne (2005). "Homologues of insecticidal toxin complex genes in *Yersinia enterocolitica* biotype 1A and their contribution to virulence." Infect. Immun. **73**(10): 6860-6867.

Thisted Lambertz, S. and M. L. Danielsson-Tham (2005). "Identification and characterization of pathogenic *Yersinia enterocolitica* isolates by PCR and pulsed-field gel electrophoresis." Appl. Environ. Microbiol. **71**(7): 3674-3681.

Thompson, J. S. and M. J. Gravel (1986). "Family outbreak of gastroenteritis due to *Yersinia enterocolitica* serotype 0:3 from well water." Can. J. Microbiol. **32**(8): 700-701.

Thomson, N. R., S. Howard, B. W. Wren, M. T. Holden, L. Crossman, G. L. Challis, C. Churcher, K. Mungall, K. Brooks, T. Chillingworth, T. Feltwell, Z. Abdellah, H. Hauser, K. Jagels, M. Maddison, S. Moule, M. Sanders, S. Whitehead, M. A. Quail, G. Dougan, J. Parkhill and M. B. Prentice (2006). "The complete genome sequence and comparative genome analysis of the high pathogenicity *Yersinia enterocolitica* strain 8081." PLoS Genet **2**(12): e206.

Toivanen, P., A. Toivanen, L. Olkkonen and S. Aantaa (1973). "Hospital outbreak of *Yersinia enterocolitica* infection." Lancet **1**(7807): 801-803.

Toma, S. and V. R. Deidrick (1975). "Isolation of *Yersinia enterocolitica* from swine." J. Clin. Microbiol. **2**(6): 478-481.

Toma, S., G. Wauters, H. M. McClure, G. K. Morris and A. S. Weissfeld (1984). "O:13a,13b, a new pathogenic serotype of *Yersinia enterocolitica*." J. Clin. Microbiol. **20**(5): 843-845.

Tomaso, H., G. Mooseder, S. A. Dahouk, C. Bartling, H. C. Scholz, R. Strauss, T. M. Treu and H. Neubauer (2006). "Seroprevalence of anti-*Yersinia* antibodies in healthy Austrians." Eur. J. Epidemiol. **21**(1): 77-81.

Tuohy, A. M., M. O'Gorman, C. Byington, B. Reid and W. D. Jackson (1999). "*Yersinia* enterocolitis mimicking

Crohn's disease in a toddler." Pediatrics **104**(3): e36.

Tuori, M. R. and V. Valtonen (1983). "[*Yersinia enterocolitica* outpatient epidemic]." Duodecim(99): 706-711.

Van Damme, I., D. Berkvens, J. Bare and L. De Zutter (2013). "Influence of isolation methods on the occurrence of plasmid-carrying *Yersinia enterocolitica* serotype O:3 in slaughter pig tonsils, faeces and carcass surface swabs." Int. J. Food Microbiol. **164**(1): 32-35.

Van Noyen, R., J. Vandepitte and G. Wauters (1980). "Nonvalue of cold enrichment of stools for isolation of *Yersinia enterocolitica* serotypes 3 and 9 from patients." J. Clin. Microbiol. **11**(2): 127-131.

Van Noyen, R., J. Vandepitte, G. Wauters and R. Selderslaghs (1981). "*Yersinia enterocolitica*: its isolation by cold enrichment from patients and healthy subjects." J. Clin. Pathol. **34**(9): 1052-1056.

Van Ossel, C. and G. Wauters (1990). "Asymptomatic *Yersinia enterocolitica* infections during an outbreak in a day-nursery." Eur. J. Clin. Microbiol. Infect. Dis. **9**(2): 148.

Vasala, M., S. Hallanvuo, P. Ruuska, R. Suokas, A. Siitonen and M. Hakala (2013). "High frequency of reactive arthritis in adults after *Yersinia pseudotuberculosis* O:1 outbreak caused by contaminated grated carrots." Ann. Rheum. Dis.

Viboud, G. I. and J. B. Bliska (2005). "*Yersinia* outer proteins: role in modulation of host cell signaling responses and pathogenesis." Annu. Rev. Microbiol. **59**: 69-89.

Virtanen, S., R. Laukkanen-Ninios, P. Ortiz Martinez, A. Siitonen, M. Fredriksson-Ahomaa and H. Korkeala (2013). "Multiple-Locus Variable-Number Tandem-Repeat Analysis in Genotyping *Yersinia enterocolitica* Strains from Human and Porcine Origins." J. Clin. Microbiol. **51**(7): 2154-2159.

Wagner, S. J. (2004). "Transfusion-transmitted bacterial infection: risks, sources and interventions." Vox Sang. **86**(3): 157-163.

Wang, X., Z. Cui, D. Jin, L. Tang, S. Xia, H. Wang, Y. Xiao, H. Qiu, Q. Hao, B. Kan, J. Xu and H. Jing (2009). "Distribution of pathogenic *Yersinia enterocolitica* in China." Eur. J. Clin. Microbiol. Infect. Dis.

Wang, X., W. Gu, Z. Cui, L. Luo, P. Cheng, Y. Xiao, L. Tang, B. Kan and H. Jing (2012). "Multiple-locus variable-number tandem-repeat analysis of pathogenic Y*ersinia enterocolitica* in China." PLoS One **7**(5): e37309.

Wang, X., Y. Li, H. Jing, Y. Ren, Z. Zhou, S. Wang, B. Kan, J. Xu and L. Wang (2011). "Complete genome sequence of a *Yersinia enterocolitica* "Old World" (3/O:9) strain and comparison with the "New World" (1B/O:8) strain." J. Clin. Microbiol. **49**(4): 1251-1259.

Wauters, G., S. Aleksic, J. Charlier and G. Schulze (1991).

"Somatic and flagellar antigens of *Yersinia enterocolitica* and related species." Contrib. Microbiol. Immunol. **12**: 239-243.

Wauters, G., M. Janssens, A. G. Steigerwalt and D. J. Brenner (1988). "*Yersinia mollaretii* sp nov and *Yersinia bercovieri* sp nov, formerly called *Yersinia enterocolitica* biogroups 3A and 3B." Int. J. Syst. Bacteriol. **38**: 14-21.

Wauters, G., K. Kandolo and M. Janssens (1987). "Revised biogrouping scheme of *Yersinia enterocolitica*." Contrib. Microbiol. Immunol. **9**: 14-21.

Weagant, S. D. (2008). "A new chromogenic agar medium for detection of potentially virulent *Yersinia entero-colitica*." J. Microbiol. Methods **72**(2): 185-190.

Weissfeld, A. S. and A. C. Sonnenwirth (1980). "*Yersinia enterocolitica* in adults with gastrointestinal disturbances: need for cold enrichment." J. Clin. Microbiol. **11**(2): 196-197.

Wesley, I. V., S. Bhaduri and E. Bush (2008). "Prevalence of *Yersinia enterocolitica* in market weight hogs in the United States." J. Food Prot. **71**(6): 1162-1168.

Weynants, V., V. Jadot, P. A. Denoel, A. Tibor and J. J. Letesson (1996). "Detection of *Yersinia enterocolitica* serogroup O:3 by a PCR method." J. Clin. Microbiol. **34**(5): 1224-1227.

Weynants, V., A. Tibor, P. A. Denoel, C. Saegerman, J. Godfroid, P. Thiange and J. J. Letesson (1996). "Infection of cattle with *Yersinia enterocolitica* O:9 a cause of the false positive serological reactions in bovine brucellosis diagnostic tests." Vet. Microbiol. **48**(1-2): 101-112.

Wong, K. K., M. Fistek and R. R. Watkins (2013). "Community-acquired pneumonia caused by *Yersinia enterocolitica* in an immunocompetent patient." J. Med. Microbiol. **62**(Pt 4): 650-651.

Wren, B. W. (2003). "The *yersiniae*--a model genus to study the rapid evolution of bacterial pathogens." Nat Rev Microbiol **1**(1): 55-64.

Young, G. M., J. L. Badger and V. L. Miller (2000). "Motility is required to initiate host cell invasion by *Yersinia enterocolitica*." Infect. Immun. **68**(7): 4323-4326.

Zhang, L., J. Radziejewska-Lebrecht, D. Krajewska-Pietrasik, P. Toivanen and M. Skurnik (1997). "Molecular and chemical characterization of the lipopoly-saccharide O-antigen and its role in the virulence of *Yersinia enterocolitica* serotype O:8." Mol. Microbiol. **23**(1): 63-76.

Zheng, H., Y. Sun, S. Lin, Z. Mao and B. Jiang (2008). "*Yersinia enterocolitica* infection in diarrheal patients." Eur. J. Clin. Microbiol. Infect. Dis. **27**(8): 741-752.

Zheng, H., Y. Sun, Z. Mao and B. Jiang (2008). "Investigation of virulence genes in clinical isolates of *Yersinia entero-colitica*." FEMS Immunol. Med. Microbiol. **53**(3): 368-374.

Zheng, H., J. Wang, Y. Sun and B. Jiang (2007). "Clinical isolation

and characterization of *Yersinia enterocolitica* in China using real-time PCR and culture method." Digestion **75**(4): 199-204.

Zink, D. L., J. C. Feeley, J. G. Wells, C. Vanderzant, J. C. Vickery, W. D. Roof and G. A. O'Donovan (1980). "Plasmid-mediated tissue invasiveness in *Yersinia enterocolitica*." Nature **283**(5743): 224-226.

Appendix

Bacterial strains used in the study. Strain originated from human fecal samples if not otherwise indicated. Strains isolated in this study are marked bold.

Species	BT/ST	Strain	Isolation	Article
Y. bercovieri		**FE 80153**	11/01/2006	I, II
Y. bercovieri		**FE 80392**	03/02/2006	I, II
Y. bercovieri		**FE 80555**	22/02/2006	I, II
Y. bercovieri		**FE 80587**	21/02/2006	I, II
Y. bercovieri		**FE 80870**	16/03/2006	I, II
Y. bercovieri		**FE 80940**	31/03/2006	I, II
Y. bercovieri		**FE 80989**	29/03/2006	I, II
Y. bercovieri		**FE 81021**	04/04/2006	I, II
Y. bercovieri		**FE 81190**	24/04/2006	I, II
Y. bercovieri		**FE 81202**	22/04/2006	I, II
Y. bercovieri		**FE 81357**	09/05/2006	I, II
Y. bercovieri		**FE 81523**	01/06/2006	I, II
Y. bercovieri		**FE 81634**	04/06/2006	I, II
Y. bercovieri		**FE 81717**	16/06/2006	I, II
Y. bercovieri		**FE 81864**	04/07/2006	I, II
Y. bercovieri		**FE 81945**	05/07/2006	I, II
Y. bercovieri		**FE 81998**	17/07/2006	I, II
Y. bercovieri		**FE 82011**	14/07/2006	I, II
Y. bercovieri		**FE 82215**	31/07/2006	I, II
Y. bercovieri		**FE 82240**	26/07/2006	I, II
Y. bercovieri		**FE 82396**	02/08/2006	I, II
Y. bercovieri		**FE 83121**	09/09/2006	I, II
Y. bercovieri		**FE 83591**	10/11/2006	I, II
Y. enterocolitica	1A	**FE 80044**	05/01/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80045**	09/01/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80053**	02/01/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80054**	03/01/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80055**	03/01/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80079**	03/01/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80082**	05/01/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80088**	05/01/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80138**	02/01/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80140**	03/01/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80150**	16/01/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80152**	12/01/2006	I, II, IV, V

Y. enterocolitica	1A	**FE 80175**	17/01/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80178**	19/01/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80183**	19/01/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80202**	17/01/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80216**	19/01/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80217**	25/01/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80255**	18/01/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80257**	24/01/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80259**	24/01/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80260**	24/01/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80261**	21/01/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80287**	20/01/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80303**	03/02/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80313**	01/02/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80328**	27/01/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80335**	01/02/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80355**	07/02/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80367**	30/01/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80377**	06/02/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80384**	13/02/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80385**	13/02/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80418**	10/02/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80419**	15/02/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80420**	15/02/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80429**	12/02/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80430**	12/02/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80444**	21/02/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80446**	20/02/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80447**	20/02/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80468**	20/02/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80469**	17/02/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80470**	16/02/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80533**	27/02/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80556**	22/03/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80588**	22/02/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80646**	07/03/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80647**	27/02/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80648**	10/03/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80681**	06/03/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80727**	06/03/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80728**	06/03/2006	I, II, IV, V

Y. enterocolitica	1A	**FE 80729**	07/03/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80757**	14/03/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80766**	14/03/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80775**	20/03/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80791**	15/03/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80792**	15/03/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80800**	21/03/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80819**	20/03/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80837**	21/03/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80890**	27/03/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80891**	20/03/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80931**	22/03/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80936**	28/03/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80937**	28/03/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80938**	26/03/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80947**	29/03/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80950**	02/04/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80960**	28/03/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80961**	03/04/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80983**	29/03/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80987**	01/04/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80990**	30/03/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81004**	04/04/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81005**	10/04/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81006**	01/04/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81007**	31/03/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81009**	06/04/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81016**	07/04/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81017**	06/04/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81018**	06/04/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81019**	03/04/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81020**	05/04/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81051**	18/04/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81058**	13/04/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81059**	18/04/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81077**	18/04/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81078**	12/04/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81079**	14/04/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81086**	19/04/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81096**	22/04/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81097**	15/04/2006	I, II, IV, V

Y. enterocolitica	1A	**FE 81124**	14/04/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81158**	24/04/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81159**	25/04/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81171**	27/04/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81172**	27/04/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81173**	26/04/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81174**	25/04/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81188**	27/04/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81189**	25/04/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81198**	28/04/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81200**	29/04/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81225**	04/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81226**	05/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81227**	04/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81228**	27/04/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81229**	27/04/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81230**	26/04/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81250**	04/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81251**	28/04/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81256**	09/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81257**	09/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81258**	08/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81278**	11/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81279**	11/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81280**	04/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81281**	11/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81296**	10/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81346**	13/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81350**	03/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81351**	04/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81358**	09/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81364**	10/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81365**	11/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81366**	12/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81367**	15/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81379**	17/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81406**	17/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81407**	16/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81408**	15/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81410**	24/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81411**	21/05/2006	I, II, IV, V

Y. enterocolitica	1A	**FE 81416**	12/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81417**	17/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81418**	13/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81429**	22/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81435**	26/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81450**	19/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81452**	19/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 8145 4**	22/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81498**	29/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81507**	31/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81508**	30/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81522**	02/06/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81527**	02/06/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81533**	18/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81534**	23/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81535**	24/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81536**	19/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81550**	26/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81551**	28/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81556**	05/06/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81569**	31/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81570**	06/06/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81571**	05/06/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81593**	02/06/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81594**	01/06/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81595**	01/06/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81596**	29/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81601**	30/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81602**	27/05/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81604**	05/06/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81622**	05/06/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81623**	06/06/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81624**	06/06/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81629**	08/06/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81636**	14/06/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81637**	12/06/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81649**	15/06/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81651**	16/06/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81668**	18/06/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81718**	13/06/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81728**	14/06/2006	I, II, IV, V

Y. enterocolitica	1A	**FE 81732**	13/06/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81747**	16/06/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81773**	21/06/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81774**	21/06/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81816**	19/06/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81825**	22/06/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81826**	28/06/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81834**	26/06/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81835**	28/06/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81836**	27/06/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81865**	29/06/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81866**	29/06/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81870**	30/06/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81875**	29/06/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81883**	30/06/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81890**	29/06/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81891**	05/07/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81892**	05/07/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81906**	10/07/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81907**	02/07/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81908**	02/07/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81922**	12/07/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81926**	04/07/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81944**	05/07/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81964**	06/07/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81965**	14/07/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81997**	11/07/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 81999**	18/07/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 82005**	14/07/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 82009**	13/07/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 82010**	13/07/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 82052**	16/07/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 82101**	25/07/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 82121**	26/07/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 82172**	18/07/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 82173**	27/07/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 82185**	19/07/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 82200**	24/07/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 82229**	01/08/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 82321**	06/08/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 82325**	02/08/2006	I, II, IV, V

Y. enterocolitica	1A	**FE 82326**	31/07/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 82397**	02/08/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 82405**	09/08/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 82435**	09/08/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 82477**	07/08/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 82539**	11/08/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 82540**	11/08/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 82541**	16/08/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 82555**	21/08/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 82583**	21/08/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 82584**	14/08/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 82585**	15/08/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 82586**	15/08/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 82587**	15/08/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 82588**	23/08/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 82621**	24/08/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 82623**	18/08/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 82624**	17/08/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 82687**	22/08/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 82813**	25/08/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 82814**	28/08/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 82892**	29/08/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 82904**	31/08/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 82957**	05/09/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 82958**	12/09/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 82993**	13/09/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83013**	08/09/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83053**	21/09/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83102**	18/09/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83105**	18/09/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83128**	19/09/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83208**	29/09/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83241**	06/10/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83244**	09/10/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83247**	28/09/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83248**	05/10/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83249**	05/10/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83276**	06/10/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83300**	11/10/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83301**	11/10/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83314**	08/10/2006	I, II, IV, V

Y. enterocolitica	1A	**FE 83335**	13/10/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83336**	11/10/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83367**	17/10/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83420**	23/10/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83433**	28/10/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83469**	27/10/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83470**	27/10/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83546**	30/10/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83549**	01/11/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83553**	06/11/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83554**	06/11/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83555**	06/11/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83571**	01/11/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83580**	31/10/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83590**	10/11/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83592**	30/10/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83632**	15/11/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83642**	10/11/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83643**	13/11/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83650**	15/11/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83671**	14/11/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83672**	11/11/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83681**	07/10/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83682**	14/11/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83708**	16/11/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83726**	28/11/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83752**	28/11/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83811**	05/12/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83900**	11/12/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83923**	18/12/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83954**	12/12/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83955**	13/12/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83970**	27/12/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83984**	18/12/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83985**	20/12/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 83986**	22/12/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 84010**	18/12/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 84017**	31/12/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 84031**	28/12/2006	I, II, IV, V
Y. enterocolitica	1A	**FE 80919**	20/03/2006	I, II, IV, V
Y. enterocolitica	2/O:9	**FE 80256**	24/01/2006	I, II, III, V

Y. enterocolitica	2/O:9	**FE 82000**	18/07/2006	I, II, III, V
Y. enterocolitica	2/O:9	**FE 83011**	10/09/2006	I, II, III, V
Y. enterocolitica	2/O:9	**FE 83088**	21/09/2006	I, II, III, V
Y. enterocolitica	2/O:9	**FE 83117**	27/09/2006	I, II, III, V
Y. enterocolitica	3/O:3	**FE 81568**	05/06/2006	I, II, III, V
Y. enterocolitica	3/O:3	**FE 83494**	30/10/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 80016**	04/01/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 80017**	05/01/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 80037**	02/01/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 80061**	10/01/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 80098**	13/01/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 80128**	16/01/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 80139**	09/01/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 80354**	09/02/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 80382**	08/02/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 80476**	24/02/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 80534**	27/02/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 80535**	28/02/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 80665**	09/03/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 80666**	12/03/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 80686**	10/03/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 80703**	10/03/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 80704**	08/03/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 80735**	15/03/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 80869**	28/03/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 80967**	30/03/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 81008**	06/04/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 81076**	11/04/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 81094**	11/04/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 81095**	12/04/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 81123**	20/04/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 81249**	03/05/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 81265**	08/05/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 81298**	05/05/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 81299**	09/05/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 81361**	16/05/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 81442**	27/05/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 81567**	06/06/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 81606**	10/06/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 81630**	10/06/2006	I, II, III, V

Y. enterocolitica	4/O:3	**FE 81652**	15/06/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 81654**	14/06/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 81679**	13/06/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 81758**	27/06/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 81852**	29/06/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 81921**	10/07/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 81962**	13/07/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 82162**	27/07/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 82205**	30/07/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 82241**	07/08/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 82404**	31/07/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 82407**	04/08/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 82473**	09/08/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 82474**	11/08/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 82476**	04/08/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 82491**	15/08/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 82545**	15/08/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 82560**	21/08/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 82731**	24/08/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 83012**	15/09/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 83014**	15/09/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 83031**	15/09/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 83073**	18/09/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 83084**	19/09/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 83099**	25/09/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 83100**	19/09/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 83223**	05/10/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 83299**	09/10/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 83306**	13/10/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 83312**	16/10/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 83313**	13/10/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 83387**	25/10/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 83434**	30/10/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 83458**	20/10/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 83581**	31/10/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 83593**	08/11/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 83631**	08/11/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 83725**	28/11/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 83753**	29/11/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 83905**	15/12/2006	I, II, III, V
Y. enterocolitica	4/O:3	**FE 84053**	28/12/2006	I, II, III, V

Y. enterocolitica	NBT	**FE 81455**	28/05/2006	I, II, IV, V
Y. enterocolitica	NBT	**FE 83264**	10/10/2006	I, II, IV, V
Y. frederiksenii		**FE 80758**	09/03/2006	I, II
Y. frederiksenii		**FE 80860**	20/03/2006	I, II
Y. frederiksenii		**FE 80151**	05/01/2006	I, II
Y. frederiksenii		**FE 80258**	13/01/2006	I, II
Y. frederiksenii		**FE 80477**	24/02/2006	I, II
Y. frederiksenii		**FE 80988**	03/04/2006	I, II
Y. frederiksenii		**FE 81042**	03/04/2006	I, II
Y. frederiksenii		**FE 81297**	01/05/2006	I, II
Y. frederiksenii		**FE 81451**	19/05/2006	I, II
Y. frederiksenii		**FE 81653**	14/06/2006	I, II
Y. frederiksenii		**FE 81716**	16/06/2006	I, II
Y. frederiksenii		**FE 81884**	30/06/2006	I, II
Y. frederiksenii		**FE 82008**	13/07/2006	I, II
Y. frederiksenii		**FE 82401**	08/08/2006	I, II
Y. frederiksenii		**FE 82402**	08/08/2006	I, II
Y. frederiksenii		**FE 82403**	09/08/2006	I, II
Y. frederiksenii		**FE 82446**	10/08/2006	I, II
Y. frederiksenii		**FE 82475**	04/08/2006	I, II
Y. frederiksenii		**FE 82561**	16/08/2006	I, II
Y. frederiksenii		**FE 82702**	18/08/2006	I, II
Y. frederiksenii		**FE 82730**	25/08/2006	I, II
Y. frederiksenii		**FE 82732**	24/08/2006	I, II
Y. frederiksenii		**FE 83166**	18/09/2006	I, II
Y. frederiksenii		**FE 83179**	23/09/2006	I, II
Y. frederiksenii		**FE 83457**	27/10/2006	I, II
Y. frederiksenii		**FE 83724**	16/11/2006	I, II
Y. frederiksenii		**FE 83773**	27/11/2006	I, II
Y. frederiksenii		**FE 83810**	27/11/2006	I, II
Y. intermedia		**FE 82216**	31/07/2006	I, II
Y. intermedia		**FE 80200**	12/01/2006	I, II
Y. intermedia		**FE 80253**	22/01/2006	I, II
Y. intermedia		**FE 80254**	22/01/2006	I, II
Y. intermedia		**FE 80517**	13/02/2006	I, II
Y. intermedia		**FE 80939**	27/03/2006	I, II
Y. intermedia		**FE 81400**	11/05/2006	I, II
Y. intermedia		**FE 81521**	30/05/2006	I, II
Y. intermedia		**FE 82660**	24/08/2006	I, II
Y. kristensenii		**FE 80334**	30/01/2006	I, II
Y. kristensenii		**FE 80982**	24/03/2006	I, II

Y. kristensenii		**FE 81441**	18/05/2006	I, II
Y. kristensenii		**FE 81650**	15/06/2006	I, II
Y. kristensenii		**FE 83456**	27/10/2006	I, II
Y. kristensenii		**FE 83862**	01/12/2006	I, II
Y. mollaretii		**FE 80304**	25/01/2006	I, II
Y. mollaretii		**FE 80518**	21/02/2006	I, II
Y. mollaretii		**FE 81453**	22/05/2006	I, II
Y. mollaretii		**FE 81621**	05/06/2006	I, II
Y. mollaretii		**FE 81696**	12/06/2006	I, II
Y. mollaretii		**FE 81963**	05/07/2006	I, II
Y. mollaretii		**FE 82209**	27/07/2006	I, II
Y. mollaretii		**FE 82747**	28/08/2006	I, II
Y. mollaretii		**FE 83914**	01/12/2006	I, II
Y. mollaretii		**FE 83956**	19/12/2006	I, II
Y. mollaretii		**FE 83958**	12/12/2006	I, II
Y. mollaretii		**FE 84032**	21/12/2006	I, II
Y. rohdei		**FE 80667**	06/03/2006	I, II
Y. rohdei		**FE 82589**	09/08/2006	I, II
Y. enterocolitica	4/O:3	IHI250239	04/12/2003	III
Y. enterocolitica	4/O:3	IHI250242	10/12/2003	III
Y. enterocolitica	4/O:3	HI250243	15/12/2003	III
Y. enterocolitica	4/O:3	HI250244	16/12/2003	III
Y. enterocolitica	4/O:3	HI250246	17/12/2003	III
Y. enterocolitica	4/O:3	HI250247	12/12/2003	III
Y. enterocolitica	4/O:3	HI250248	12/12/2003	III
Y. enterocolitica	4/O:3	HI250249	11/12/2003	III
Y. enterocolitica	4/O:3	HI250251	16/12/2003	III
Y. enterocolitica	4/O:3	HI250252	16/12/2003	III
Y. enterocolitica	4/O:3	HI250254	22/12/2003	III
Y. enterocolitica	4/O:3	HI250255	23/12/2003	III
Y. enterocolitica	4/O:3	HI250256	23/12/2003	III
Y. enterocolitica	4/O:3	HI250257	29/12/2003	III
Y. enterocolitica	4/O:3	HI250258	30/12/2003	III
Y. enterocolitica	4/O:3	HI250260	02/01/2004	III
Y. enterocolitica	4/O:3	HI250261	30/12/2003	III
Y. enterocolitica	4/O:3	HI250265	20/01/2004	III
Y. enterocolitica	4/O:3	HI250266	20/01/2004	III
Y. enterocolitica	4/O:3	HI250267	20/01/2004	III
Y. enterocolitica	4/O:3	HI250271	13/01/2004	III
Y. enterocolitica	4/O:3	HI250272	15/01/2004	III
Y. enterocolitica	1A	FE 94338 (Food)		IV